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PREFACE

A three-day symposium on "Amoebiasis and other Intestinal Infections" was held in the Central Drug Research Institute, Lucknow, from the 16th to the 18th November 1959. Contributions had been invited from Indian and foreign laboratories, University institutions and research organizations. Over fifty delegates from different research centres in India, USA, Great Britain, France, S Africa and Switzerland participated in the symposium. Twentyseven papers on different aspects of the chemotherapy of amoebiasis and three on allied infections were presented.

The symposium was inaugurated by Shri V V Giri, Governor of Uttar Pradesh on the 16th November 1959. The Chief Minister of U P, Dr. Sampurnanand, who could not be present due to a previous engagement, sent a message emphasizing the need for such a symposium to evolve methods for the eradication of amoebiasis. Professor M S Thacker, in his opening remarks, stressed the need for the development of new and more effective methods of therapy as existing methods of treatment have not proved satisfactory. Lively and illuminating discussions followed almost every paper, the contributions from the assembled scientists were of a high scientific standard and helped to clarify many important points of interest in the problem of amoebiasis.

Different sessions were presided over by Col S S Bhatnagar, Hony Professor of Microbiology, St Xavier's College, Bombay, Dr M L Dhar, Assistant Director, CDRI, Lucknow, and Dr A S Sanjivi, Retired Professor of Medicine, Medical College, Madras. The symposium concluded with an address by Dr B B Bhatia, Professor of Medicine, K G Medical College, Lucknow. I am very much indebted to them for their valuable help and guidance.

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Proceedings of the Symposium
on Amoebiasis and other Intestinal Infections
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INAUGURAL ADDRESS

SHRI V V GIRI

Governor of Uttar Pradesh

I consider it an honour to have been asked to inaugurate this symposium on 'Amoebiasis' this morning and I extend a cordial welcome to the distinguished scientists who have come here to participate in it

Amoebiasis, a type of dysentery, is prevalent all over the world. Its incidence is more so in our country both in acute and chronic forms. The main causes of this disease are insanitary living conditions, bad water supply, unwholesome and infected food. In its chronic type, the disease often recurs making the individual totally invalid and is believed to be incurable.

We find references to this malady in our ancient Ayurvedic literature written by Charaka and Sushruta. Even to-day we find the age old remedies recommended by them being followed by the people. But the last word on a suitable and efficacious remedy is yet to be said and it would be unscientific to accept a cure blind-foldedly simply because we have inherited it from the past. It is highly necessary to keep an open mind and do research on these native drugs as much as is being done on modern drugs and antibiotics.

While we may continue to take steps to cure those sufferings from this disease, it is equally our bounden duty to formulate measures that would help us in eradicating it totally. The foremost thing is to emphasize upon the people the importance of clean living and of following proper hygienic rules.

Another reason for the high percentage of the prevalence of this disease is due to the lack of elementary wants to fulfil the needs of the individual for maintaining the health of an individual, such as nutritious food, enough clothing and a little house in healthy surroundings and timely medical attention whenever necessary are to be provided. If this is adopted as the basis for our programmes, many of the diseases would fast disappear. Efforts should be made to realise the fundamental rights guaranteed by the Constitution at the earliest possible opportunity. In this task, every section of the community should come forward and co-operate to achieve the cherished goals.

The aetiology of this disease was discovered as early as 1875, but still a large number of problems remain to be solved. The knowledge we have gathered is only fragmentary, just a handful compared to the ocean of unravelled mysteries. A symposium in which eminent experts are participating is bound to contribute much to an understanding of the many issues that confront us. That this should

I have much pleasure in inaugurating this symposium and I wish your deliberations every success

MESSAGE FROM CHIEF MINISTER UTTAR PRADESH

It would have given me much pleasure to be present at this conference. The discussion of the subject is naturally bound to be of a highly technical nature, but I am sure even a layman like me would have found the symposium interesting and instructive. Previous engagements however, prevent me from accepting the invitation to attend it.

Amoebiasis is a subject of great interest not only to the specialist but to the average citizen as well particularly to residents of tropical countries like India and methods of its eradication deserve the fullest attention of medical men as well as the State. It has been studied from very ancient times. The aetiology, diagnosis and treatment of diarrhoea, dysentery and other diseases of the intestinal region have received very detailed treatment in ancient medical literature which definitely mentions that ailments of this kind may among other reasons be due to action of *Arimi* (अरिम्) that is minute living organisms. The instructions given in these books are based on a long experience of conditions in the climatic and other conditions obtaining in this country and deserve to be seriously studied by medical men even to-day. As is well known Ayurveda does not stop merely at what might be called the immediate cause namely the amoebae. It tries to go a step further back. It asks the question why a particular patient should be liable to attacks of this kind while others living in the same conditions seem to enjoy a complete immunity? The answer it gives stems from the theory of *Tridosha*. I need not stress this point further.

I have no doubt that the deliberations at this symposium will lead to the enunciation of such practical steps as can be adopted with a view to curb the disease. Curative medicine is useful of course, but what is most needed is preventive medicine and instruction to the common man in such rules of health as can be easily understood and followed by the average Indian citizen keeping in mind the social and economic conditions prevailing in the country, specially in our villages.

SAMPURNANAND

OPENING REMARKS

PROF M S THACKER

Secretary Ministry of Scientific Research and Cultural Affairs and Director General Council of Scientific and Industrial Research New Delhi

(Read by Dr B Mukerji Director CDRI)

I am happy to have this privilege of associating myself with this symposium and to offer a few remarks as a preliminary to initiate further and more technical

greater attention and further investigative work in this field of amoebic and therefore when Dr Mukerji request to organize an all and also suggested that the g international participation if possible I am glad to see that this objective has been reached to some extent and we have with us some experts from different countries abroad I am confident that this joint deliberation will be beneficial for all concerned in the development of newer drugs for amelioration and treatment of this type of infection

mosquito and anti larval measures adopted on a world scale and also the evolution of newer and more effective anti malaria remedies Amoebiasis is a disease that has been called a subtle murderer and as such has not received as intensive attention of world research workers as it deserves It is cosmopolitan in distribution Specially prevalent in India Indo China China and the Philippines it is common throughout North Central and South Africa Southern United States South America and the West Indies A Russian physician F Losch in St Petersburg in 1875 found amoebae in stools of patients and in ulcers of the colon and was apparently able to transmit the disease to a dog The pioneering work of specially the German workers like Schaudinn and Hartmann and of the British workers like Wenyon and Dobell has clearly shown that several species of amoebae occur in the intestinal canal of human beings Out of these *Entamoeba histolytica* is pathogenic the other species of amoebae are harmless

So little information is available regarding the inherent pathogenicity of

amoebic infection Beltran in 1948 pointed out on the basis of 169 surveys in 43 countries representing more than 350 000 human beings that the global infection of *E histolytica* is 13 per cent Europe has 10 per cent while the Americans show an average of 12 per cent infection The highest rate was found

for Asia and Africa 16 and 17 per cent respectively. In India very little detailed and careful survey has been made so far.

The earlier researches gave rise to great controversies concerning the role of *E. histolytica* in the causation of amoebiasis. Many of these conflicts were eliminated by experimental data from human and animal experiments. *E. histolytica* can live in the lumen of the large intestine and can invade the mucosa and form ulcers. From these ulcers the amoebae frequently move to the liver causing liver abscesses. Lung and brain abscesses are rare. The later controversies are concerned with the question whether *E. histolytica* can live in 'carriers' as a commensal or whether it is always pathogenic or whether the presence of certain types of bacteria is necessary for pathogenicity. The demonstration by Boeck and Drbohlav in 1925 of a method of cultivation of *E. histolytica* and the discovery of suitable laboratory animals, especially young rats by Jones in 1946 in Britain for the production of experimental amoebiasis are bound to throw light on these important questions.

The fragmentary state of knowledge regarding the physiology of *E. histolytica* is clearly reflected in the largely empirical approach to the search for effective amoebicides. In 1926 Wenyon said that emetine hydrochloride and emetine bismuth iodide were the only drugs considered effective in amoebiasis. Since that time a large number of amoebicidal agents including antibiotics have been in current use but most cures that have been followed for considerable periods of post treatment have relapsed. As metastatic lesions may also be present in the

chronic course of the disease. The ideal chemotherapeutic agent has yet to be discovered.

New methods of therapy are needed because of dissatisfaction with the currently available drugs. It is to be hoped that the papers and discussions in this symposium may throw light on them. I wish your deliberations every success.

CERTAIN ASPECTS OF THE PROBLEM OF AMOEBIASIS

■ N SINGH

From the Central Drug Research Institute, Lucknow

Amoebiasis is one of the most serious disease caused by protozoa and is

disease to a dog. Even after 84 years of the discovery of the aetiology of this disease there are a number of problems in amoebiasis which need solution. I shall deal here with only a few of them.

The fragmentary state of our knowledge regarding the physiology of amoebae is clearly reflected in the largely empirical approach in the search for drugs to cure amoebiasis. Anderson *et al* (3) have listed a total of 27 agents in current use including 4 preparations of emetine, 7 aromatic arsenicals, 3 iodoquinolines, 7 antibiotics, 1 kurchi alkaloid, 1 German product (Gavano)

and 1 acridine (Rivanol), 2 bismuth compounds and chloroquin. Most "cures" that have been followed for considerable periods of post treatment have relapsed. This suggests that the antiamoebic drugs discovered, so far, have little or no effect on the cystic stage of *E. histolytica*. When the effect of the drug disappears, the amoebae come out of the cysts, feed on suitable bacteria present in human intestine, multiply and cause relapse. This seems to be the case in chronic amoebiasis for which there is no effective cure. It is, therefore, important to discover drugs that should be, in addition to having amoebicidal property, cysticidal or prevent amoebae forming cysts or make the amoebae come out of the cysts. This question has not, so far, seriously attracted the attention of biologists and chemists engaged on the chemotherapy of intestinal amoebiasis. It seems to me that it is exceedingly difficult, if not impossible, to discover agents that will be cysticidal and will not kill or damage the host. Very little, indeed, is known regarding the factor or factors causing encystation in protozoa. Moreover, it

that a strain of *Aerobacter aerogenes* produces in an actively proliferating culture, in a synthetic medium, a thermolabile excystment factor. More recently, Singh, Mathew and Anand (5) discovered that aqueous extracts of common human intestinal bacteria like *A. aerogenes* and *Escherichia coli* cause excystment of viable sterile amoeba cysts. A part of the excystment inducing activity was due to the presence of amino acids, some of which have been identified with the aid of paper partition chromatography. Some chemically pure amino acids and a few

The factors responsible for the virulence or invasiveness of *E. histolytica* are indeed very little understood. It has been conclusively established (6) that about 80% of the infections are carrier cases. In such temperate zone as Great Britain the disease does not seem to occur, although 10% of the population of some countries parts of riers and dysentery or due to

some newly acquired virulent or invasive strain. Dew (8) has recently raised the question "Is the concept put forward by Brumpt (9) correct and the amoebae of the temperate zones are a different species, *E. dispar*, and that of the tropics, *E. histolytica*?" If that

important in determining whether the large number of contact carrier cases should be treated or not for amoebic infection.

The question of host parasite relations in infections with *E. histolytica* has been reviewed excellently by Hoare and Neal (10). They have drawn the conclusion "A vast literature has accumulated, dealing with clinical course

of these views is sup admitted that this question as experimental animal parable conditions to man

There is some evidence of the strain differences from acute and contact "carrier" cases. It has been demonstrated conclusively by Neal (12, 13), Neal and

Vincent (14) and Singh *et al* (15) that strains from 'carrier' cases are practically non invasive to rats, though they are able to infect this host. Strains from acute cases are not able to do this as in the case of rat. Pooni *et al* (16) have

differ in virulence but it is not clear whether these differences are innate in the strains. Recently, Singh (17) has discovered that two strains of *F. histolytica* one isolated in culture from temperate zone (EA strain) and the other from semi tropical zone at Lucknow from 'carrier' cases, become virulent and produce extensive ulceration in the caeca of rats when the amoebae are fed with cholesterol in culture. Similarly, these non invasive strains give rise to extensive ulceration when the animals are fed with cholesterol in olive oil, either before or after intracaecal inoculation of amoebae. When the amoebae from extensive ulceration are suspended in normal saline and injected into rats, they retain their virulence through two successive passages in the absence of cholesterol. Neal (18) was unable to make EA strain of *E. histolytica* virulent by the change in bacterial flora or liver passage in hamsters or by making it encyst in culture. Strains from acute cases, that had become avirulent by keeping the amoebae in culture for a long time, could be made virulent to rats by hamster liver passage.

The unreliability of certain methods used in the laboratory diagnosis of amoebiasis has created much confusion. There is much controversy regarding the value of indirect cultural and serological methods. The task of detecting

Chaudhuri and Chaudhuri (20) from Calcutta based on their careful work of several years. Comparatively low percentage of infection in Lucknow may be due to the climatic factor. Lucknow has a dry and hot climate for most of the year and so the survival time of cysts of *E. histolytica* outside human body for infecting new hosts may be much less than in humid climates of Calcutta, Madras and Bombay.

REFERENCES

- 1
- 2
- 3
- 4
- 5
- 6
- 7
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- 9

10	Mathew, S, Singh, B N and Misra, S S (1956), <i>J Indian Med Assoc</i> , 51 , 230	1,
11		
12		
13		
14		
15		
16		

J Trop Med Hyg, **5**: 1000

- 17 Singh, B N (1959), *J Sci Industr Res*, **18C**: 166
- 18 Neal, R A (1957), *Trans R Soc Trop Med Hyg*, **51**: 313
- 19 Mathew, S, Singh, B N and Misra, S S (1956), *J Indian Med Assoc*, **26**: 13
- 20 Chaudhuri, R N and Chaudhuri, M N R (1946), *Indian Med Gaz*, **81**: 230

DISCOVERY OF DRUGS FOR AMOEBIASIS

I HAWKING

From the National Institute for Medical Research, London

to make it more active and less toxic, and eventually a compound may be discovered which is sufficiently promising to test in man

The first stage of this process is the "screening test" which is designed to pick out the few active compounds from the large number of inactive compounds

many other infections also. The history of the effective compounds which have been discovered in the past emphasizes the need to test a wide range of compounds (because often activity has been discovered in unexpected quarters) and to test compounds on a wide range infections (because compounds synthesized for one purpose have been found active in quite a different manner). The critical evaluation of "active compounds" which may be discovered in this way is left to more elaborate tests

Testing in vitro

Turning now to the specific infection of amoebiasis (due to *Entamoeba histolytica*) in which this Institute is particularly interested, we may say that the simplest and most direct method of testing compounds is to examine their action upon the amoebae *in vitro*. The amoebae are suspended in a suitable medium (Balamuth's egg-yolk infusion liver extract) in glass tubes containing suitable concentrations of the new compounds, and they are incubated at 37°C for 24

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compounds which are active *in vivo* are inactive *in vitro* and would, therefore, be missed by a purely *in vitro* test. Accordingly, most laboratories supplement *in vitro* tests with *in vivo* tests

In vivo tests

At the Central Drug Research Institute, tests *in vivo* have been developed in rats. Young rats weighing 25-30g are anaesthetized and the caecum is brought to the surface. A suspension containing about 50,000-100,000 amoebae in 0.5 cc fluid is injected into the caecum, and the wound is sewn up. The rats are treated with the unknown compound by mouth for 5 successive days, starting on the

dose a

rats

rats a

controls, the lesions should be severe (score 4), i.e. the wall of the caecum should be much thickened and ulcerated, the caecal content should consist mostly of mucus without faeces, and many amoebae should be present in the contents and in scrapings from the wall. Each drug is given to 4 rats, and the control group contains 6 rats, if the findings in

of activity the compound is tested again. If many compounds are to be tested it is

at the first test and to repeat all doubtful results, rather than to use large groups which theoretically might yield more reliable results but which would consume much compound and reduce the number of tests which could be made. Most of the compounds submitted to test are inactive and can be discarded after the first trial. If a compound shows activity then more detailed tests are required to assess its activity in comparison with that of other compounds. Elaborate investigations of toxicity are carried out and eventually (if fortune is extremely favourable) the results may justify a clinical trial.

All this work
are many and succ

inspite of disappointments, money to pay for the large expenses involved, and luck. One often wonders whether luck is not the most important ingredient of all.

THERAPEUTIC TRIALS IN AMOEBIASIS

R. ELSDON DEW

From the Amoebiasis Research Unit, Durban, South Africa

The Amoebiasis Research Unit* has for many years been engaged in the trial of various therapeutic substances in the treatment of amoebiasis in Africans in Durban. In these people, for some as yet unknown reason, this disease is particularly prevalent: some 3000 cases presenting each year at one hospital. Liver abscess, diffuse peritonitis and other complications are not infrequently seen.

*Sponsored by the following bodies: The Council for Scientific and Industrial Research, The Natal Provincial Administration, The University of Natal, and the United States Public Health Service (Grant E 1592).

From this plethora of material patients with acute amoebic dysentery of average severity are selected for therapeutic trial. Each patient must fulfil the following criteria

- 1 Actively motile haematophagous amoeba must be found in stools or scrapings from ulcers
- 2 Ulcers must be visible on sigmoidoscopy
- 3 There must be neither complications nor co existing disease

In this way, the clinical material is uniform an essential criterion in the comparison of different therapies

Sigmoidoscopy is performed on admission and at frequent intervals thereafter, usually on the 5th, 10th, 20th and 27th days after the commencement of treatment. Stool examination is done at the same intervals and daily if ulceration persists after the end of treatment. Patients are hospitalised for a minimum of 28 days and are encouraged to return for examination at monthly intervals thereafter. At the 5th, 10th, 20th and 27th days patients are classified in the following categories

- 1 Success—symptom free, ulcers healed and no parasites demonstrable
- 2 Probable failure—persistent ulceration with no parasites demonstrable in stools or scrapings
- 3 Parasitic failure—amoeba still present with or without open ulceration

Once an efficient method of therapy had been established, those patients whose condition was deteriorating and those showing ulceration and trophozoites after the completion of the course of therapy under trial were removed and given the most efficient therapy known

Initially 50 cases were treated by each form of therapy, but, once the more efficient therapies had been established, it was no longer necessary to proceed to 50 cases unless the new drug proved to be competitive. The results of some of the trial therapies are given in the Table I which indicates final assessment at 27 days. Those drugs or combinations which are not likely to be of interest have been omitted from the list

DISCUSSION

Study of list of trials indicates a process of evolution. In the acute form of amoebic colitis, the old stand by emetine, though controlling symptoms, failed to remove parasites in a quarter of the cases, even when given in a dose usually regarded as toxic. The higher dose of 15 gr. was no more effective than 10 grains. Dihydroxyquinoline was not significantly better and there was

Carbarsone though showing some activity was not really competitive, and chinofon only cured some 70% of cases and its side effects were unpopular.

It was the investigation of the 'shot gun' therapy of Hargreaves that opened a new vista. When this therapy was split into its components it was realized

TABLE II
Therapeutic trials

Trial no	Therapy	Daily dosage	Days of therapy	No of cases	Assessment at 27 days (%)			Remarks
					Success	Probable failure	Parasitic failure	
1	Emetine	gr 1	0.15	50	50	22	28	
2	D iodoxyquinoline	tabs 9	0.20	50	58	18	24	
3	Emetine	gr 1	0.15	58	64	24	2	
4	D iodoxyquinoline	18 g	0.20	62	75	23	1	
5	Emetine	gr 1	0.10	62	75	23	1	
6	D iodoxyquinoline	18 g	0.20	50	80	14	6	
7	Emetine Bismuth Iodide	gr 1/2	0.10	50	80	14	6	
8	D iodoxyquinoline	18 g	0.10	45	58	31	11	
9	Emetine	gr 1	0.10	45	58	31	11	
10	Carbanone	0.3 mega	0.7	59	95	5	0	Note that anti amoebic therapy follows ant bacterial
11	Penicillin	150 g	0.7	49	90	8	2	Ant amoebic and anti bacterial therapies simultaneous
12	Succinyl sulphathiazole	18 g	0.20	49	38	10	1	
13	Emetine	gr 1	0.10	50	92	2	6	
14	D iodoxyquinoline	0.3 mega	0.20	57	56	12	34	
15	Succinyl sulphathiazole	15 g	0.20	55	46	16	38	
16	Penicillin	0.3 mega	0.20	10	10	40	50	
17	Chloroquine diphosphate	15 g	0.1	10	10	40	50	
18	Carbanone	10 g	2.5	10	10	40	50	
19	Emetine	0.5 g	6.15	10	10	40	50	
20	Carbanone	gr 1/4	0.10	10	50	10	40	
21	Emetine bismuth iodide (whole)	gr 1/4	0.10	68	44	6	50	Pills appeared in stools
22	Phenobarbitone	gr 1	0.10	51	94	4	2	
23	Chlortetracycline	10 g	0.15	51	94	4	2	

TABLE I (Contd.)

Trial no.	Therapy	Daily dosage	Days of therapy	No. of cases	Assessment at 27 days (%)			Remarks
					Success	Probable failure	Parasitic failure	
23	Bismuth glycyl-l-aspartate	1.5 g	0-10	21	28	10	62	
24	Chiniofon	1.5 g 3.0 g	0.5 4-10	49	71	2	27	
25	Emetine (control)	gr 1	0-10	25	56	28	16	
26	Emetine	gr 1	0-10	49	80	18	2	
	Carbarsone	gr viii	0-10					
	Emetine bismuth iodide	3.0 g	11-20					
	Chiniofon	1.5 g 3.0 g	11-13 14-20					
27	Oxytetracycline	1.0 g	0-15	51	92	2	6	
28	Neomycin	40000 units	stat	12	33		67	
29	Bacitracin	40000 units	stat	11	73	9	18	
30	Neomycin	40000 units 20000 units	0-10 stat	12	75	8	17	
	Bacitracin	20000 units	0-10					
31	Emetine bismuth iodide (crushed)	gr iii	0-10	53	70	9	21	
	Phenobarbitone	gr i	0-10					
32	Entamide	5 g	0-10	10	30	30	40	
33	Entamide benzoate	5 g	0-10	10	40	30	30	
34	Streptomycin	2.0 g	0-10	17	12	—	88	29% cyst passers after therapy
35	Chloramphenicol	1.0 g	0-15	5	40	—	60	
36	Chloramphenicol (enteric coated)	1.0 g	0-15	8	38	—	62	
37	Chloramphenicol	4.0 g 2.0 g	0-3 4-12	16	62	—	38	
38	Polymyxin	0.4 g	0-10	7	14	—	86	
40	Smearobidin	50 mg	0-10	8	38	—	62	
41	Smearobidin	0.2 g	0-10	■	47	—	33	

TABLE I (Contd.)

Trial no	Therapy	Daily dosage	Days of therapy	No. of cases	Assessment at 27 days (%)		Remarks
					Success	Probable failure	
42	Furazolidin	40 mg	0.10	7	—	—	100
43	Furazolidin	200 mg	0.10	47	70	4	26
44	Furodil	60 mg	0.10	11	18	—	82
45	Emetine bismuth iodide (enteric)	gr iii	0.10	41	80	5	15
48	Food only	—	—	10	—	—	100
51	Penicillin	0.2 mega	0.10	—	—	—	—
	Diiodohydroxyquinoline	18 g	0.20	53	79	11	10
	Emetine	gr i	0.10	—	—	—	—
53	Oxytetracycline residue	40 g	0.10	9	67	—	31
54	Oxytetracycline intra muscular	0.2 g	0.10	5	40	20	40
55	Chlortetracycline	80 mg	0.10	10	60	—	40
■	Streptomycin residue	40 g	0.10	10	10	10	80
57	Chlortetracycline residue	40 g	0.10	13	77	—	23
59	Tetracycline	10 g	0.10	59	97	—	3
60	Turamycin	0.5 g	0.5	10	40	—	60
61	Diphtheria	2.5 g	0.10	43	74	7	19
62	Entam de (enteric coated)	50 g	0.10	10	—	40	60
63	Erythromycin	20 g	0.10	21	71	14	10
64	Chlortetracycline	100 mg	0.10	—	—	—	—
	Diiodohydroxyquinoline	18 g	0.20	48	94	2	4
	Chloroquine	0.5 g	0.15	—	—	—	—
65	Spiramycin	20 g	0.10	■	83	7	6
66	Novobiocin	10 g	0.10	■	30	20	50
67	Novobiocin	20 g	0.10	9	33	11	56
69	Acintazole	300 mg	0.10	13	31	8	61
							One case (4%) developed liver abscess
							One case (5%) showed toxicity
							30% cyst passers after therapy
							1g 200 mg chlorte tetracycline 15% cyst passers
							1g 110 mg oxytetracycline

and parasitic cure indicated the importance of the part played by the bacteria associated in the genesis and maintenance of the disease.

This led to an antibiotic approach, and the tetracyclines gave a success rate unprecedented by any other single drug therapy. Apparently, the wider the anti-bacterial spectrum the more effective the therapy, whether this was achieved by a single drug or by a combination. Seemingly, the varied nature of the bacterial species sustaining the amoeba implies the use of a therapy likely to hit as many different species as possible.

A series of antibiotics were tried, with some surprising results. Chloramphenicol, for example, converted a high proportion of cases into cyst passers, leading to some speculation as to the role of the surviving bacterial species in the maintenance of the commensal state of the amoebae.

Antibiotics aimed directly at the amoeba were tried. Iumagilin which *in vitro* had shown extreme promise, failed in the human, and was relatively ineffective even in toxic doses.

The high cost and the dangers of the use of massive doses of tetracycline led to the use of smaller doses and though these were not as dramatic as the more drastic therapy they were effective. However the development during anti-bacterial therapy of such complications as liver abscess, and the continued occurrence of some relapses indicated that this approach is not the complete answer. It is apparent that in our present state of knowledge the therapeutic attack must be aimed at (i) the amoeba in the lumen, (ii) the amoeba devouring the bowel wall and (iii) the amoeba in the tissues. The obvious drugs are a

the most
effective
ever, to
1

of the tetracycline
this dosage the d
to potentiate the
to use somewhat higher dosages of tetracycline

However, our results so far merely indicate the necessity for continued study, possibly in the direction of some anti-metabolite to divert that essential substance which the amoeba obtains from bacteria in the bowel and from man in the tissues.

Trials continue

SUMMARY

A review is given of the therapeutic trials in acute ulcerative amoebic colitis, carried out by the Amoebiasis Research Unit in Durban, South Africa. Over eighty forms of therapy have been tried.

ACKNOWLEDGMENT

The extensive clinical work was carried out by Dr F G Armstrong, Dr R J Marot, Dr A J Wilmot, Dr N R Pooler and Dr S J Powell.

These trials would not have been possible without the co-operation of the Director of the Hospital Services, Dr J Parker, and of the Superintendent of King Edward VIII Hospital, Dr S Disler.

I wish to thank the various drug companies for the supply of many of the materials under test.

EXPERIMENTAL CHEMOTHERAPY OF AMOEBIASIS

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From the Calcutta School of Tropical Medicine, Calcutta

Drugs showing anti amoebic properties *in vitro* are usually evaluated in experimental animals before subjecting them to clinical trial. In the past the testing methods utilized experimental infections in kittens, dogs or monkeys or natural amoebiasis in monkeys. Owing to practical difficulties smaller mammals are now commonly used for the purpose. Of the rabbits, guinea pigs, hamsters

made between the lumen of the caecum and the exterior either on the ventral (Fig. 2) or on the dorsal aspect (Fig. 3) of the animals. A set of washer and a nut fixes the tube to the parietal wall and a cap at the outer end prevents leakage of caecal contents. About the tenth day of operation the animal is ready for experiment; it lives normally with the tube *in situ* for an indefinite period. Utilizing this technique some observations have been made firstly on the result of inoculation through the tube and secondly on the effect of amoebicidal drugs also introduced through the tube. The aim and object had been to see if the

clinical relapse

per
daily
and

numbers indicating onset of infection which occurred usually between the 5th and 12th day of inoculation. More than half of the animals became infected

Lower part of the colon also

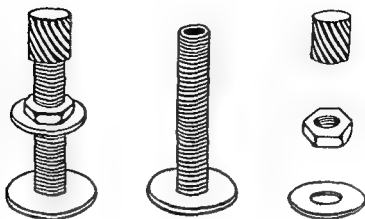


Fig. 1. Fixtrew with its different parts.



Fig. 2. Guinea pig with ventral fixation.

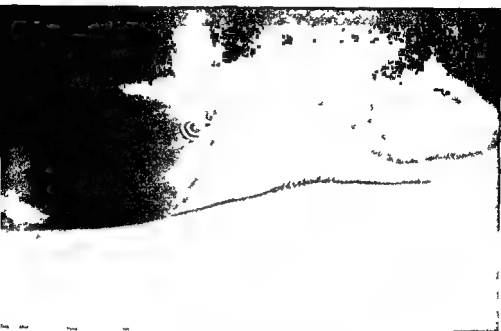


Fig 3 Guinea pig with dorsal fixation

In another series of animals an amoebicidal drug was administered at about the onset of infection and the progress was assessed by daily examination of caecal contents for amoebae. Seven drugs were arbitrarily used in 63 infected animals as indicated below by the following preliminary trials.

1 *Emetine bismuth iodide*—It was given to 7 infected guinea pigs in dosage of 8 mg once daily for 10 days. In 3 of them the caecal content became negative on the 4th, 6th and 7th day of treatment, one however died 11 days after the course. The remaining 4 animals died between the 5th and 10th days of treatment with the persistence of infection. In the 2 animals apparently responding to therapy the caecal content was positive 9 and 16 days after completion of treatment. They also died soon after.

2 *Carbarsone*—It was given in daily dosage of 25 mg to 4 animals. The

weeks

Another 4 infected animals were given a smaller dosage of 15 mg daily for 10 days. Three failed to respond and died between the 6th and 10th days of treatment. In the fourth the caecal content became negative on the 4th day, the animal remained well for 4 weeks after completion of the course. Thereafter, *F. histolytica* reappeared in the caecal content.

3 *Kurchi bismuth iodide* (prepared in the Chemistry Department) was tried in 12 infected guinea pigs the dose being 60 mg once daily for 10 days. In 7

the amoebae disappeared from the caecal content between the 2nd and 7th days of treatment, but reappeared between 15 and 48 days after the treatment. In the remaining 5 they persisted throughout the period.

4 *Ipecac bismuth iodide* (prepared in the Chemistry Department) was given to 12 animals in dosage of 6 mg once daily for 10 days. In 4 of them, the caecal content was negative on the 3rd, 6th, and 8th day of treatment respectively. Two of them died on the 7th and 8th days with liquid motions, although the caecal content was free from amoebae and the other 2 became positive again on the 27th and 35th days after completion of therapy. All the other 8 animals died between the 5th and 10th days of therapy with persistence of amoebae in the caecal content. Five of them also had loose motions with frank liquid character of caecal contents.

5 *Mebinol V* (Carlo Erba) — This drug (ethoxy-ethyl phenoxy nitrobenzyl

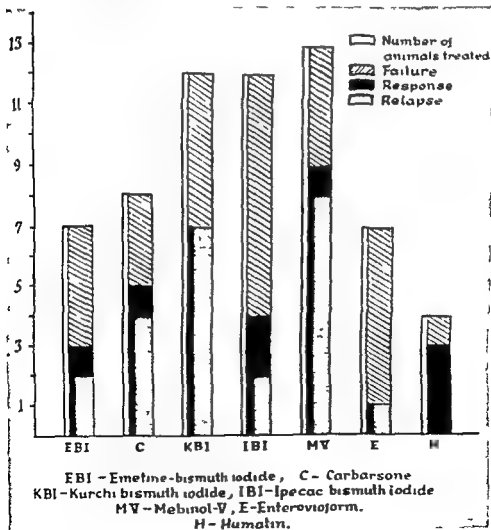


Fig. 4. Graph showing results of screening amoebicidal drugs.

amoebae in the caecal content

6. *Enterovioform* (Ciba) was given to 7 infected guineapigs in a dosage of 125 mg once daily. In 6 of them it had no effect as was evident from the persistence of amoebae in the caecal contents and all of them died within 5 and 11 days. One became negative on the 5th day but was positive again 11 days after the course.

7. *Humatin* (*Paromomycin*)—This antibiotic obtainable from cultures of *Streptomyces* was tried in 4 infected guineapigs. The results are as follows:

The remaining animal died on the 5th day. All the 3 animals responding to the treatment remained well with negative reports throughout the period of observation for a month.

The accompanying graph (Fig. 4) represents the preliminary chemotherapeutic response *in vivo* to the seven drugs in the doses mentioned.

Without attempting to draw any conclusion on the relative efficacy and toxicity of the particular drugs from the above *in vivo* study, it appears that the experimental device evolved promises to be of value not only in observing the natural course of *E. histolytica* infection in guineapigs but also in assessing the effect of different compounds on amoebae in the colon. The animals that would respond and survive the infection with disappearance of parasites from the caecal content may also be followed up for possible recurrence, chronicity and other studies. The work is in progress in the clinical Research Unit, Indian Council of Medical Research.

Our thanks are due to Shri P. Ghose for technical assistance.

AN APPROACH TO THE CHEMOTHERAPY OF AMOEBIASIS

M L DHAR

From the Central Drug Research Institute, Lucknow

Dr B Mukerji gave a review of important landmarks in the chemotherapy of amoebiasis yesterday and there is one scheduled from Dr J Druey for this session of the conference. I shall refer at my remarks to the work of Dr B

way open to the workers in this important field. It is, however, probably possible to introduce a degree of rationale in this empiricism. In our laboratories, we felt that emetine and conessine, the two powerful systemically active amoebicides, may provide leads to an attack on this problem.

In an assessment of the possible mode of drug receptor association, we concluded that hydrogen bonds aided by van der Waals' associations were operative in the case of emetine and conessine. I discussed this subject in detail in an

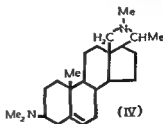
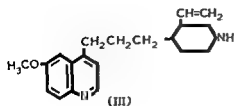
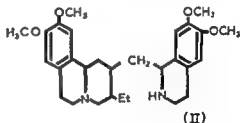
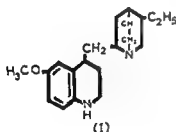
protein helix

The simple diamines bearing an electronic status and the intervening distance of the two nitrogen atoms nearly equivalent to those of emetine and conessine were shown to possess high *in vitro* activity (2). Major deviation from these conditions led to the abolition of activity. Likewise, restriction of the mobility

activity was dependent also on the steric disposition of the related molecular

molecules by these forces
without the aid of electrostatic
the interacting molecules in

tive agent. It is
between desoxy
ethyl, piperidyl
the rupture of the



9 Chloro 9 desoxyquinine, obtained by the treatment of quinine monohydrochloride with thionyl chloride or phosphorus pentachloride, gave on reductive dehalogenation with hydrogen in presence of palladium charcoal, 9 desoxydi-hydroquinine which was reduced further by the method of Pyman for the reduc-

For obtain
quinine was
and Rhodc
corresponding

experimental

bioreceptor

quinolines (12). These latter compounds were prepared with a view to provid-
ing alternate centres for chelation in the quinoline molecule and to find out if
they retained antiamoebic activity. Since 8 hydroxyquinolines are held by the
4, 5, 6
tive both

Others are still under test

I may suggest that the activity of the 8 methoxy quinolines, quinaldines,
quinazolines and quinoxalones would perhaps not be considered to be dependent
on chelation unless demethylation preceeds actively in the biophase, and that
these may act by a different mechanism possibly one that governs the activity of
chloroquin

REFERENCES

1. Dhar M. L. p. 7 (1959) *Pro. Sympos. on Chemotherapy* (Central Drug Research Institute Lucknow) p. 7
2. Kashyap B. S. (1956) *7. C. Ind. D. 1956*
- 3.
- 4.
- 5.

- 6 Pyman F. L. (1917), *J Chem Soc*, **111**: 419
 - 7 Singh, B. N. and Sharma R. (1958) *Proc Symposium on Chemotherapy*, Central Drug Research
 Institute, Lucknow, 1957
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IN VITRO APPROACHES TO CHEMOTHERAPY OF AMOEBIASIS

P. S. KAUSHIK

From the Central Drug Research Institute, Lucknow

determining whether or not a compound has any specific anti amoebic effect

METHODS

The chief drawback in conducting *in vitro* tests is that so far it has not been possible to cultivate *E. histolytica* in the test tube free from other living cells and this fact poses several problems. On a closer examination, it will be worth-

(1) *Test medium* The choice of test medium in a drug testing programme is of great importance. A prerequisite is that the medium should be able to support the growth of amoebae as well as the concomitant associates. Numerous media exist but the simplest possible media are preferable in order to minimise the side-effects of the ingredients. Egg extracts (egg eluates) (2) provide a simple medium for conducting drug tests and all fluid (monophasic) media are generally employed in comparison to biphasic media because of ease in handling and to ensure uniform distribution of drugs. The presence of special substances (free proteins like serum) (3) —SH group (4,5) and pH in the medium (6,7) influences the drug activity. Reardon and Rees (8) have done the evaluation of drugs upon *E. histolytica* without overlay serum. The present trends have also led to the development of all fluid essentially synthetic medium of

accepted that intestinal amoebae require is requirement is either met by special 1) or the reducing conditions are produced by the physicochemical conditions created by the microbial associates

distinguish between direct and indirect activity of chemical agents. Duration of incubation is also an important factor in antiamoebic action and sufficient attention should be paid to the period of drug exposure. The *in vitro* sensitivities

TABLE I
In vitro screening methods employed in various laboratories

No	Test medium compositions	Incubation duration (hr)	Inoculum		Criteria of endpoint	
			Associates	Amount (per cc)	Direct examination & subcultivation	Associates
1	Serum Ringer (pre conditioned)	72	Monobacterial		Direct examination	Methylene blue
2	Serum Ringer	24	Polybacterial		Direct examination & sub-cultivation	—
3	Liver egg yolk	48	(a) Monobacterial (b) Polybacterial	3 × 10 ⁴	Direct examination & sub cultivation	(a) O/R potential (b) Population
4	Egg eluate	(a) 24	Polybacterial	3 × 10 ⁴	Direct examination	—
		(b) 24-48	Polybacterial	5 × 10 ⁴	Direct examination & sub cultivation	—
		(c) 24-48	Polybacterial	Massive	Direct examination	Use of drug fast bacteria
5	Liver protense peptone	48	Monobacterial	3 × 10 ⁴	Direct examination & sub cultivation	(a) O/R potential (b) Respiration
6	Trypticase-thio glycolate blood (preconditioned)	72	<i>T. cruzi</i>	1	Direct examination	motility
7	Egg-salt Locke (biphase)	(a) 48	<i>T. cruzi</i>		Direct examination & sub cultivation	Population
		(b) 48	<i>T. cruzi</i>		Direct examination & sub-cultivation	Population
		(c) 48	Monobacterial	500	Direct examination & sub cultivation	Population
8	Serum liver extract (micromethod)	(a) 24	Polybacterial		Direct examination	—
		(b) 48	Polybacterial	3 × 10 ⁴	Direct examination	—
		(c) 24-48 (horizontal)	Polybacterial	10 amoebae / 1/6 high power	Direct examination	—

of four strains of *E. histolytica* have been studied by de Cameri (11) after various periods of incubation. The effect of drug incubation period also depends upon the nature of test substance. Some highly effective direct acting amoebicides (cf actidione) have much less effect at 24 hr than at 48 hr. However, a longer period of incubation entails the risk of confusing antibacterial activity with antiamoebic activity. This risk is worth taking in the case of synthetic compounds. In our experience most of the synthetic compounds tested in the laboratory showed hardly any difference in dilution endpoints at 24 and 48 hr intervals.

TABLE 1 (continued)

No	Reference	Amoebicidal endpoints of some selected drugs ($\mu\text{g/cc}$)			
		Emetine	Vioform	Carbarsone	Newer compounds
1	Dobell (14)	5	—	—	—
	Goodwin (24)	10	—	100	—
	Fulton (25)	5	—	—	—
	Neal (26)	5	—	—	10 (Mantonide and Entamide)
	Kradolfer (27)	10	2500	1200	60 (Entobex)
2	Kaushiva (16-18)	8	—	—	—
3	Balamuth (10-22)	5	100	—	0.01 (fumagilin)
4	(a) Thompson (28)	4.5	15	1000	—
	(b) Kaushiva (29-30)	3.9	10	—	—
	(c) Thompson (1, 2)	10	—	—	3.9 (Humatin)
5	Anderson (13)	10	100	2000	—
6	Phillips (12)	5	—	—	—
7	(a) Anderson (13)	—	—	—	0.1 (Fumagilin)
	(b) Bradner (31)	31.3	—	—	—
	(c) Brackett (32)	200	80	—	—
8	(a) Rawson (33)	62.5	—	—	—
	(b) Lynch (34)	3.9	—	—	0.03 (Fumagilin) 12.5 (Mantonide)
	(c) Woolfe (21)	2	1.100	—	0.01 (Fumagilin) 1.0 (Mantonide) 0.1 (Entamide)

(iii) *Inoculum* Both the quantitative (amount, age, growth phase) and qualitative (strain and species differences, culture associates) aspects of the inoculum are important. The inocula have varied quantitatively from undeter-

are taken against confusing bactericidal action with amoebicidal action. As already stated the presence of associates complicates the evaluation of amoebicidal drugs particularly in the assessment of antibiotics. Balamuth (10) used large inoculum and recorded pH and viable bacterial populations at the apparent endpoints coupled with subcultivation of all negative tubes to eliminate cases of inhibition.

The associates have varied from unknown mixed bacterial flora (polybacterial) to single species of bacteria (monobacterial) and in some cases have been the

fact that it is more resistant than bacteria to most antibiotics and other amoebicidal drugs. The methods employed for assessing the susceptibility of the associates include the study of populations and effect on motility. Biochemical indices have been measured in terms of reducing activity of the medium (O.R. potential) (10) reduction of indicator dyes (14) and oxygen consumption of the associates (13).

Ultimately, drugs under test are evaluated in terms of amoebicidal activity and it is highly desirable to standardize the following parameters in testing: (a) numbers of amoebae in inoculum, (b) criterion of amoebicidal endpoint for complete kill, (c) probable effect of agents upon the bacterial flora. It is necessary to employ subcultivation into preconditioned normal culture medium and to preferably employ monobacterial cultures. Amoebicidal endpoints are

involved in the tests and lack information on the range of sublethal concentrations in cultures have been analysed. The numbers of surviving amoebae can be determined more accurately and the errors in ascertaining death of a culture are eliminated. The extent and nature of damage can also be assessed by exposing them to sublethal concentrations and examining

The choice of amoebae other than the causative agent as test objects for rapid and large scale screening of amoebicidal compounds depends on the assumption that if the sensitivities of *E. histolytica* against different amoebicides could be

and *Didascalus thorntoni*) and *E. histolytica* has been made (16). A similar comparative study on the response of *E. madseni* and *E. histolytica* to several drugs has been made by McConnachie (17) and de Carneri (18) and they have discussed the significance of their studies on the use of *E. madseni* for chemotherapeutic research in amoebiasis. This may prove fruitful by drawing a parallel with the approaches leading to the development of the antimalarial Daraprim.

LABORATORY STUDIES

being amoebicidal at low concentrations and for its direct action as it is not antibacterial even at high concentrations, for these reasons it has served as a standard by which the effect of other drugs is measured. Emetine also shows differential action against *E. histolytica* in comparison to other species of intestinal amoebae (10). In Table II, the dilution endpoint activity of emetine and conessine

TABLE II
Sensitivities of amoebae to emetine and conessine

Experiment No	<i>E. histolytica</i>	Emetine (μ g/cc)		<i>N. gruberi</i>	<i>E. histolytica</i>	Conessine (μ g/cc)		
		<i>S. rustelli</i>	<i>D. thomasi</i>			<i>S. rustelli</i>	<i>D. thomasi</i>	<i>N. gruberi</i>
1	■	500	250	63	63	■	16	31
2	8	125	125	31	31	8	4	31
3	4	250	125	250	63	■	4	31
4	8	125	125	31	63	■	4	4
5	8	31	125	63	31	16	125	4
6	2	500	125	500	31	16	31	4
7	8	31	63	250	63	4	4	31
8	4	125	63	31	31	8	4	31
9	8	125	125	63	63	8	8	4
10	8	125	125	63	63	■	4	31
Likely amoebicidal endpoint	8	125	125	31	63	8	4	31
Mean amoebicidal endpoint	6	194	125	135	50	10	10	20

against different species of amoebae are given. The noteworthy difference is

activity is strangely limited to species belonging to the genus *Entamoeba* and appears to be inactive *in vitro* against *Hartmannella castellani*.

A comparison of amoebicidal activity of a number of compounds against different species of amoebae is given in Table III. The susceptibilities of amoebae

TABLE III

Antibacterial end points ($\mu\text{g/lcc}$) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines



Series	Compd no	R	R	E. histolytica	S. russelli	D. thornton	N. gruberi
(M)							
(N)							
(Q)							
(P)							

TABLE III (Contd.)

Amoebicidal end points ($\mu\text{g}/\text{cc}$) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines

Series	Compd no	R	III	E. histolytica	S. rustelli	D. discoideum	N. gruberi
(R)							
		$\text{H}_2\text{O}_2\text{AsH}_2\text{C}_4$	$\text{CO}-\text{NH}$				
		$\text{CH}-\text{C}-\text{NR}$					
			S				
	R1	$\text{C}_6\text{H}_5\text{CH}_2(\alpha)$		250	1000	1000	125
	R2	$\text{C}_6\text{H}_5\text{CH}_2(\beta)$		1000	1000	1000	250
	R3	$\text{C}_6\text{H}_4\text{Cl}(\beta)$		500	500	250	125
	R4	$\text{C}_6\text{H}_4\text{Cl}(\beta)$		125	500	1000	1000
	R8	$\text{C}_6\text{H}_4\text{COOH}(\alpha)$		1000	500	500	1000
	R9	$\text{C}_6\text{H}_4\text{COOH}(\alpha)$		>1000	1000	>1000	>1000
	R10	$\text{C}_6\text{H}_4\text{COOH}(\beta)$		>1000	1000	>1000	>1000
	R11	$\text{C}_6\text{H}_5(\alpha)$		250	500	500	250
(A)							
		$(\alpha\text{-B})_3\text{N}(\text{CH}_2)_6\text{NHR}$					
	69	C_6H_5		16	31	31	31
	70	C_6H_5		16	31	16	31
	71	C_6H_5		1	16	31	31
	72	C_6H_5		1	2	4	8
	73	C_6H_5		16	8	8	4
	76	Benzyl		31	16	31	31
	77	$(\text{CH}_2)_6\text{Ph}$		4	16	31	8
(B)							
		$\text{---}(\text{CH}_2)_6\text{NHR}$					
	78	C_6H_5		63	250	31	1000
	79	C_6H_5		63	500	250	1000
	80	C_6H_5		63	1000	250	500
	83	C_6H_5		4	250	63	63
	84	C_6H_5		4	250	31	63
	85	C_6H_5		2	31	4	8
	86	C_6H_5		2	8	4	2

TABLE III (Contd.)
 Amoebicidal end points ($\mu\text{g/sec}$) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines

Series	Compd no	R	R'	E. histolytica	S. russelli	D. thoracis	N. gruberi
(C)	87	Ph		16	16	31	16
	88	$-\text{C}_6\text{H}_4\text{OCH}_3$ (o)		16	4	8	16
	89	$-\text{C}_6\text{H}_4\text{OCH}_3$ (p)		31	63	31	63
	90	Benzyl		8	8	16	8
	91	$(\text{CH}_2)_4\text{Ph}$		8	250	125	250
(D)	100	$n\text{C}_{12}\text{H}_{25}$		4	8	8	2
	102	$-\text{C}_6\text{H}_4\text{OCH}_3$ (p)		16	16	16	8
	104	Benzyl		125	63	III	III
	106	$(\text{C}_6\text{H}_5)_2\text{NH}-$		8	1000	1000	1000
	107	$(n\text{C}_8\text{H}_{17})_2\text{NH}-$		16	500	125	16
	108	$(n\text{C}_6\text{H}_{13})_2\text{NH}-$		8	63	16	63
	109	$(n\text{C}_4\text{H}_9)_2\text{NH}-$		16	250	31	250
	110			63	1000	1000	1000
	111			31	1000	500	500

E. histolytica and 3 species of free-living amoebae against certain amines and arsenic derivatives of thiazolidone and thiazole show that free-living amoebae among themselves show a somewhat parallel behaviour in their response to the various compounds but their amoebicidal endpoints are generally different as compared to *E. histolytica*. However, correlation in the activity of some series of compounds is observed. For instance, various amoebae exist in the thiazolidone compounds (Table IV). It could be, perhaps, that thiazole ring is the molecular component active against all the amoebae. Among the polymethylene diamines, the morpholino and piperidino substituted nonanes show a significant level of correlation while butyl and disubstituted nonanes show no correlation.

TABLE IV

Correlation between sensitivities of Entamoeba histolytica and free living amoebae

Series	<i>S. russelli</i>	<i>D. thornton</i>	<i>N. gruber</i>
M	0.9999*	0.8849	0.9941
H	0.9923*	0.9998*	0.9638*
Q	-0.3421	0.3575	-0.2694
P	0.4619	0.1395	0.4014
R	-0.1059	-0.1067	-0.5713
A	0.3250	0.1952	0.3593
B	0.6840*	0.7135†	0.8803†
C	0.9990*	0.9990*	0.9999*
D	0.5513	0.5325	0.5226

*S significant at 5% level

†S significant at 1% level

In vitro methods bring out elegantly the relation between chemical structure and amoebicidal activity and provide suitable leads to the chemist for future synthesis of compounds besides eliminating less active compounds in an early stage of a screening programme. However, every testing procedure has its limitations and *in vitro* methods are not considered adequate in the case of antibiotics and insoluble compounds. Some of the insoluble benzacridine derivatives tested in our laboratory showed no activity in *in vitro* tests but were mildly active in *in vivo* tests against rats (Table V). In such cases, endpoints arrived at are not unequivocal and should be scrutinised carefully. Another disadvantage inherent in these tests is that sometimes it is not possible to differentiate between specific amoebicidal action and general protoplasmic toxicity.

CONCLUSIONS

In spite of some complexities and drawbacks *in vitro* methods have a definite role to play in the critical evaluation of amoebicidal drugs. They are direct and simple requiring small amount of test compounds. Also, they are relatively less expensive and are rapidly completed. The use of *in vitro* techniques has often

TABLE V
Amoebicidal activity of some benzacridine derivatives

Compd no	Nomenclature	In vitro minimal amoebicidal conc (µg/cc)	Oral dose (mg/kg) daily/6 days	In vitro activity*		Remarks	
				Average score amoebae	Appearance		
				Scraping of wall	Contents	Wall	Contents
AFMC 1	7 <i>p</i> -Chloroanilino benz/C/acridine HCl	100	300	2.5	0.7	3	3
AFMC 2	7 <i>p</i> -Toluidino benz/C/acridine HCl	100	300	1.7	1.7	1.7	0.7
AFMC 3	7 <i>p</i> -Bromoanilino benz/C/acridine HCl	100	300	2	2	1.7	1.2
AFMC 4	12 <i>p</i> -Iodoanilino benz/A/acridine salicylate	100	300	3	3	2.6	3.2
AFMC 5	12 <i>m</i> -Iodoanilino benz/A/acridine salicylate	100	300	1	1	1	0.5
AFMC 6	12 <i>p</i> -Anilino benz/A/acridine salicylate	10-100	300	1.2	1.2	2	1.5
				Control	4.4	3.6	4

* Four rats were used in controls and 4 rats for each compound

TABLE VI

Amoebicidal activity of some phenylamines

SERIES	COMPOUND NO.	SUBSTITUENTS	IN VITRO MINIMUM AMOEBICIDAL CONCENTRATION ($\mu\text{g/ml}$)
(A)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{R}$	BP(C) 4	$-\text{CH}_3$	> 1000
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	BP(C) 5	$-\text{C}_2\text{H}_5$	500
	BP(C) 6	$-\text{C}_3\text{H}_7$	500
	BP(C) 7	$-\text{C}_4\text{H}_9$	125
	BP(C) 2	$-\text{C}_6\text{H}_{13}$	4
(B)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{R}$	BP(C) 8	$-\text{CN}$	250
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{CH}_2\text{NH}_2\text{HCl}$	BP(C) 9	$-\text{C}_2\text{H}_5$	250
	BP(C) 10	$-\text{C}_3\text{H}_7$	250
	BP(C) 11	$-\text{C}_4\text{H}_9$	125
	BP(C) 1	$-\text{C}_6\text{H}_{13}$	4
(C)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{R}$	BP(C) 3	$-\text{C}_2\text{H}_{11}$	8-16
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{NH}_2\text{HCl}$			
(D)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{R}$	CNK 1	$-\text{C}_2\text{H}_5$	125
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{NH}(\text{CH}_2)_2\text{NH}_2\text{HCl}$	CNK 2	$-\text{C}_3\text{H}_7$	63
	CNK 3	$-\text{C}_4\text{H}_9$	31-63
	CNK 4	$-\text{C}_5\text{H}_{11}$	16
(E)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{R}$	CNK 5	$-\text{CH}_3$	16
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	CNK 6	$-\text{C}_2\text{H}_5$	16
	CNK 7	$-\text{C}_3\text{H}_7$	16
(F)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	CNK 8	$-\text{C}_2\text{H}_5$	8
	CNK 9	$-\text{C}_6\text{H}_9$	4-8
(G)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}(\text{CH}_3)\text{NH}_2\text{HCl}$	SC NK 1	$-\text{CH}_3$	> 100
	SC NK 2	$-\text{C}_2\text{H}_5$	> 100
	SC NK 3	$-\text{C}_3\text{H}_7$	> 100
	SC NK 4	$-\text{C}_4\text{H}_9$	100
	SC NK 5	$-\text{C}_5\text{H}_{11}$	> 100
	SC NK 6	$-\text{C}_6\text{H}_{13}$	10-100
(H)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	SC NK 7	$-\text{CH}_3$	> 100
	SC NK 8	$-\text{C}_3\text{H}_7$	100
(I)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	SC NK 9	$-\text{CH}_3$	100
	SC NK 10	$-\text{C}_2\text{H}_5$	100
	SC NK 11	$-\text{C}_3\text{H}_7$	100
(J)			
$\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}_2\text{NH}_2\text{HCl}$	SC NK 12	$-\text{CH}_3$	> 100
	SC NK 13	$-\text{C}_2\text{H}_5$	> 100
	SC NK 14	$-\text{C}_3\text{H}_7$	10-100
	SC NK 15	$-\text{C}_4\text{H}_9$	10

been questioned on the ground that drugs which undergo *in vivo* transformations would be missed as would also those drugs which have no activity *per se* but which help the body to overcome the infection. Such drugs are very rare and the risk involved is justifiable (21). It is to be remembered that there is no amoebicidal drug available which does not possess marked antiamoebic effect demonstrable by tests done outside the body system.

REFERENCES

- 1
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 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
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 - 27
 - 28
 - 29
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 - 32
 - 33
 - 34
- Trop Med Hyg* 3 254
- logy of Protozoa* Vol II
- armacol* 3 44
- 1 (1950) *Proc Roy Soc*

AMOEBIASIS—A CLINICAL RETROSPECT

K S SANJIVI*

The incidence of amoebiasis is most difficult to assess inasmuch as a person may have the infection and still not exhibit any symptoms whatsoever, or a patient may have highly suggestive symptoms and the laboratory demonstration of amoebic cysts in the stools may not be forthcoming.

Routine examination of the stools for cysts has been carried out in different parts of the world with very variable results. While in certain parts of the U.S.A. it has been assessed that as many as 5 to 20% of apparently healthy persons harbour cysts similar studies in Lucknow and in Madras have shown a lesser incidence of cyst passers. This is only one example of the vagaries of examinations for amoebic cysts. I am not aware of any serological or tissue sensitivity test which may perhaps find more universal application than the demonstration of the amoeba or the cyst in the stool. The complement fixation test has not been accepted as reliable.

With the limitations in laboratory diagnosis therefore it is not surprising that the clinical diagnosis of amoebiasis is apt to be exaggerated or under estimated according to the fancies of a particular physician.

CLINICAL TYPES

Acute amoebic dysentery

No detailed description of this common clinical condition is necessary. Although the differential diagnosis between severe bacillary dysentery and mild amoebic dysentery can be fairly easily made on clinical data alone such as the number of stools, the naked eye character of the stools, and the constitutional reactions, the distinction is more difficult in the average cases. The busy practitioner prescribes phthalyl sulphathiazole on hearing the patient's account of the dysentery and concludes that the case must have been one of bacillary dysentery because symptomatic improvement has been obtained with the sulphonamide. It is not sufficiently realised that the activity of the amoeba is intimately related to the bacterial content of the bowels which indeed determines the occurrence of overt symptoms and also makes any definite statement about the incubation period impossible. An antibacterial drug like sulphaguandine or phthalyl sulphathiazole is therefore quite capable of producing a temporary improvement in the clinical picture even in amoebic dysentery. But for a permanent cure, entirely different drugs must be used.

It is therefore extremely important that in every first attack of dysentery, the

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to frequent absenteeism and therefore to curtailment of the man hours put into the national productivity plans amoebiasis is as important as the other diseases mentioned

The frequent neglect of the first attack leads to the patient becoming a

be extremely difficult

(b) In children between the ages of 3 and 10 pronounced anaemia may be caused by chronic amoebic dysentery. The number of motions per day is not When reveals a to think

(c) The patient complains of constant dyspepsia with perhaps attacks of palpitation but hardly of any other symptoms suggestive of a chronic intestinal pathology. The presence of hepatic involvement undoubtedly adds to the frequency and severity of these symptoms

(d) Retention of urine has been the leading complaint in a few cases with lesions in the sigmoid and rectum. This symptom is possibly due to a reflex mechanism and among its numerous causes amoebiasis should be remembered

Complications in the liver

(a) *Hepatitis*—Amoebiasis of the liver is to my mind the commonest cause of an enlarged liver in South India. The picture of amoebic hepatitis is so

cause of a pyrexia of undetermined origin. The temperature is usually noticed in the evenings may be preceded by a chill seldom rises above 101 °F and is often not diagnosed properly

The relationship of hepatic amoebiasis to other hepatic diseases

Although the vegetative forms are permitted by lack of proper and timely treatment to reach the liver in most cases of amoebic dysentery the actual occurrence of hepatitis has been estimated to take place only in less than 10% of the subjects. Possibly the nutritional status of the liver, which suffers early in malnutrition is an important factor determining the incidence of amoebic hepatitis

rupture into the lung and produce an inflammation which may even go on to abscess formation. If, at the time of rupture, pleural adhesions are not present, a state of pyo-pneumothorax can result. Pleural effusions containing serous fluid or the typical chocolate coloured pus of amoebic infection, have been noted in certain cases.

Apart from such direct extension by actual rupture, a lymphatic extension seems also possible.

All these cases where the involvement is usually in the right base and is the result of rupture or lymphatic permeation from the liver have been designated as "secondary pulmonary amoebiasis."

inasmuch as the intestinal infection occurs first in every case.

Several types of lung involvement are possible, namely—

- (a) Amoebic pulmonary abscess without liver involvement
- (b) Pulmonary abscess separated from the hepatic process by intervening normal lung
- (c) Pulmonary abscess extending from liver abscess, which is the most frequent type
- (d) Broncho hepatic fistula with little pulmonary involvement. Here, communication between the hepatic abscess and bronchus is more direct and large permitting the evacuation of the liver abscess contents with little involvement of the pulmonary parenchyma
- (e) Perforation of hepatic abscess into the pleural cavity producing an empyema or a pyo-pneumothorax

I have seen many cases of pulmonary amoebiasis diagnosed on adequate clinical grounds. The confirmation of the diagnosis in every case was obtained by the therapeutic response of complete clearing up of all the evidences of the disease, clinical and radiological.

The various types of involvement of the lung and pleura have been referred

was rendered difficult by the fact that the sputum examination and the tomograms did not help. In view of the past history, a tuberculoma was thought of and in view of his age a carcinoma was also considered. A thoracotomy was done. The examination of the contents of the segment resected revealed amoeba.

Studies conducted in Madras

15

amoebic origin (Stanley Hospital, 1957 and 1958)

3 Reddi and Thangavelu (1) have analysed 1,011 cases occurring during the 5-year period 1941-46. Of these, 8% were in the age group 0-10 years while the greatest incidence was between 20 and 40. Males formed 82.7%. There was a higher incidence in the monsoon months. Of the 44 cases which came to autopsy, the majority showed the primary lesion starting in the caecal area. The authors had previously reported 6 cases of amoeboma (amoebic granuloma of the large intestine). During the same period 30% of all types of hepatitis were diagnosed to be of amoebic origin.

They have also reviewed a series of cases of amoebic liver abscess, 84 from the General Hospital, Madras and 40 from the King George Hospital, Vizagapatnam. The possible aetiological relationship of alcoholism is discussed. They

autopsied at Mysore and cases of cutaneous amoebiasis seen in Madras

4 Tetracyclin (250 mg. in capsules) was given 4th hourly for 10 days, along with 1 tablet of vitamin-B complex, in 6 cases of acute intestinal amoebiasis. In all of them the vegetative forms disappeared. There was clinical improvement in the dysentery in all cases.

Chlor tetracyclin, erythromycin and fumagillin are the three antibiotics which are claimed to have a direct action on the amoeba. The action of the other broad-spectrum antibiotics in controlling amoebic dysentery seems to be by the indirect method of inhibiting the activity of the bacteria in the intestinal lumen. Apparently, *Entamoeba histolytica* cannot assume pathogenicity until the bacterial flora of the intestinal tract reach a certain concentration.

5 Entamide (dichloroacet-4-hydroxy-N-methylanilide) was tried in 30 subjects passing amoebic cysts and in 33 cases with acute amoebic dysentery

the drug (2)

6 Krishnaswami and Vaidyanathan (3) have reported on a series of 20 patients treated with camoform I to 2 tablets thrice a day with meals for 5 days. Fifteen of them showed marked improvement. *F. histolytica* disappeared between the 3rd and 5th day.

of trophozoites lying free in the lumen of the intestine the amoebae in the wall of the large bowel and what have travelled to the liver and other remote areas, and lastly the cysts in the lumen of the intestine

The other usual considerations in the choice of any drug such as ease of administration freedom from toxicity and acceptability by the patient must also be taken into account

In spite of the continuous search that has been going on there is no single drug which is effective both against the trophozoites in the various sites and the cysts. It is, therefore, essential to employ a combination of amoebicides acting in different ways

The schedule of treatment that was considered to be the best until 1948, largely as the result of the experience gathered during the last war was—

Days	1- 6	Emetine injections	gr $\frac{1}{2}$ to 1
	7- 9	Carbarsone	gr 4 b d after food
	10-15	Emetine injections	gr $\frac{1}{4}$ to 1
	16-22	Carbarsone	gr 4 b d after food
	16-25	Chiniofon retention enemata	starting with III oz of 0.5% increasing by 0.5% each day up to 2.5% repeating 2.5%

It is imperative that every patient should be given the entire course of the treatment if there is to be any reasonable prospect of a permanent cure

avoided in the management of amoebiasis. In fact for over three years now, I have not used emetine at all in my practice

The schedule of treatment for the first attack of amoebic dysentery that I now employ is as follows

Days	1- 7	Tetracyclin (250 mg) or erythromycin (100 mg)	4 times a day orally
	1-14	Chloroquine diphosphate	orally 300 mg of the base b d after food for the first 2 days and then 150 mg b d
	15-28	Diadoquine entamide or equivalent	600 mg t d s after food

Cases of amoebic hepatitis and pulmonary amoebiasis have shown uniformly satisfactory response to chloroquine. In these cases of definitely established

patients have the entire course going about their work normally whereas others complain of all the symptoms enumerated above even after a day's treatment

retinopathy following chloroquine therapy refers only to cases treated with the drug for about three years continuously

The schedule of treatment indicated above is effective in acute amoebic dysentery particularly the first attack. Every now and then, however, one meets with a neglected case of chronic amoebiasis involving both the bowels and

the liver and of a fairly long duration which is extremely difficult to manage. One such patient with multiple foci of infection including skin had to be given chloroquine I M for six weeks. The patient could not tolerate the drug orally but with this long course of chloroquine, he showed remarkable improvement. It should have been obviously impossible to keep on emetine for such a long period. In some of the cases the chloroquine course had to be repeated three times with intervals of a few weeks in between before a permanent cure could be established. The exact duration of treatment therefore has to be individualised.

Prevention—The difficult problem of effectively controlling a bowel infection in a country with a poor level of general sanitation cannot be discussed here at any length. In the field of tuberculosis I feel that as medical men we cannot sit idle with folded hands waiting for the distant day when the general living

REFERENCES

1. D. C. J. T. H. M. O. S. J. J. C. S. S. S.
2. ,
- 3.
- 4.

DISCUSSION

DR P. K. GHOSH Calcutta

Q The treatment advocated by you is expensive for an average Indian patient and does not take into account the secondary infection in amoebiasis stressed yesterday.

Ans Prolonged treatment is essential to get a permanent cure and to prevent a relapse. Less expensive treatment may be less effective too.

DR SUBHIE Lucknow

Q What is the youngest age incidence in the series?
Ans 2 years

DR R. T. KARADE Hyderabad (Dn)

Q What was the radiological findings in secondary pulmonary amoebiasis?
Ans Like lung abscess

CLINICAL MANIFESTATIONS OF AMOEBIASIS

R. M. KASLIWAL AND M. M. GAMBHIR

From the S M S Medical College & Hospital, Jaipur

Amoebiasis is a disease which can have very varied manifestations. Boyers (1) reported 1961 symptoms in his 700 patients of amoebiasis. Amoebic dysentery is only one of these but to many clinicians this has erroneously become just a synonym of amoebiasis or amoebic infection. Craig (2) remarked 'The vast majority of such infections are not accompanied by dysenteric symptoms but much

or physiologically subnormal. But as Peterson (8) has remarked, "*E. histolytica* lives at the expense of the host and therefore some degree of ulceration of the intestinal mucous membrane is always present."

simulating cholecystitis and another 11 cases had burning pain in epigastrium simulating peptic ulcer. In 21 cases (11%) there were symptoms of subacute obstruction simulating abdominal tuberculosis and in 5 cases a palpable mass was felt in the regional descending colon simulating a malignant growth. These masses disappeared after successful antiamebic treatment.

Hepatic involvement—Liver involvement is the most common and the most important metastatic complication of intestinal amoebiasis. In many of these cases there may be no clinical manifestation of intestinal phase and hepatic involvement may be the first clinical manifestation. Enlarged liver was felt in slightly more than 50% of cases and about 16% cases presented as hepatitis. Liver abscess was seen in 2.2% (43 cases).

Small amount of right sided pleural effusion (which could be explained by

developed a bronchopleural fistula and the patient was bringing out anchovy sauce type of material in sputum).

These findings confirm the observations of Loeber and D. Antoni (9), Sodeman (10) and Kasliwal (6) that liver involvement is a very frequent manifestation in amoebiasis.

Anaemia—Slight degree of anaemia was very common but marked degree of anaemia was seen only in very few cases (less than 1%). This confirms the

psychitis, malaria etc.

Sigmoid colon which is also slightly tender on deep pressure. This combination has been found to be useful index for labelling cases of clinical amoebiasis.

SUMMARY AND CONCLUSIONS

Clinical records of 1934 cases of amoebiasis admitted during last 10 years to the S. M. S. Hospital, Jaipur, were studied. It was seen that most of the cases present bizarre symptoms of vague pain in abdomen and irregularity of bowel habit. Important physical findings were a slightly enlarged (tender or non-tender) liver and palpable and tender descending colon in large majority of cases.

REFERENCES

- 1 Royers L F (1933) *Calif West Med* 39 397
 2 Craig C F (1914) *Etiology diagnosis and treatment of amoebiasis* Williams & Wilkins Co
 3 Baltimore U S A
 4
 5
 6
 7
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DISCUSSIONS

DR P K GHOSH Calcutta

Q What is the pathological basis of the symptoms of cholecystic and headache syndrome in amoebiasis

Ans Headache is possibly due to dilatation of meningeal vessels by toxins (toxaemia). Diagnosis of cholecystitis was made by rapid improvement with antiamoebic drugs. About exact pathology I am not sure

Q Therapeutic test is not diagnostic of amoebiasis, for 80% of usual cases are cured by any medicine

Ans No Mere psychotherapy cannot cure or improve definite cases of amoebiasis

DR V S MANGALIK, Lucknow

Q Why is there strikingly low incidence of functional bowel disorders such as diarrhoea, dysentery and constipation etc. The total incidence being as low as 23%

Ans Chronic cases may not have any intestinal symptoms

Q Why strikingly low incidence of anaemia in these cases?

Ans In recording anaemia I have avoided cases with mild anaemia hence the chart shows a low percentage of anaemia

DR J B SRIVASTAV, Kasauli

Q Polymorphs in pleural cavity are not diagnostic of amoebic involvement, because the reaction is mononuclear in amoebic infection and the liver abscess is sterile

Ans Although theoretically polymorphs should not be in the pleural fluid,

you

Ans There was no eosinophilia in my cases

Q Did you come across any asthmatics of amoebic origin?

Ans No

Q Did you ever take the blood pressure? What are the effects on blood pressure?

Ans I do not have any definite data about blood pressure. But usually in chronic amoebiasis it is slightly lowered

DR SUBHIE, Lucknow

Q What was the youngest age of incidence in the series ?

Ans 9 months

DR S. C. MISRA, Lucknow

Q Your case records show 80% of patients had thickened sigmoid. Have you had your observation confirmed by laparotomy ?

Ans I was quite sure that there was no reflex spasm hence my clinical findings were absolutely correct. Moreover, most of the cases were confirmed by sigmoidoscopy.

DR K. V. SANJIV, Madras

Q Will you kindly tell me the percentage of cases having positive trophozoites and positive cysts in stool ?

Ans Cysts 100% trophozoites—great majority

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HEPATIC INVOLVEMENT IN AMOEBIASIS

R M KASLIWAL AND M S GAMBHIR

From the SMS Medical College, Jaipur

Clinically, hepatic amoebiasis exists in two forms (i) amoebic liver abscess and (ii) amoebic hepatitis. Amoebic liver abscess is well recognised as a definite pathological entity. The occurrence of liver abscess has been known since the time of Hippocrates who is supposed to have advocated a surgical treatment for this condition.

Councilman and Lafleur (1) probably gave the first account of pathology and pathogenesis of amoebic abscess of the liver and in a later work of Rogers (2) the aetiological and pathologic basis of this condition was established beyond doubt.

In amoebic hepatitis the picture is confusing and it has not yet received the

of *E. histolytica* to those who completely deny the existence of any diffuse lesions in the liver in amoebiasis.

(3) coined the term 'presuppurative stage' of hepatic amoebiasis for the condition the clinical picture of which he had described in his earlier work (Rogers, 2).

Since this description of Rogers, there has been a lot of controversy with regard to the existence of such a stage as a pathological entity. Two schools of thought have however emerged during this period in this field—one which

The evidence in favour of or against the existence of amoebic hepatitis has mainly come from following sources

- (i) Results of autopsy studies in cases dying of amoebiasis with or without a clinical evidence of amoebic hepatitis
- (ii) Liver biopsy studies in cases of amoebic hepatitis
- (iii) Study of liver function tests as an evidence of liver cell necrosis and diffuse lesion
- (iv)

Palmer (8) reported autopsy findings in 19 cases whose bowels showed active amoebic lesion. Microscopically there was mainly an increase in the portal connective tissue. In 9 of these cases there was a definite periportal fibrosis. In some of these patchy areas of fibrosis were also seen.

Craig (9) also found similar areas of fibrosis in the liver tissue of cases dying of amoebic dysentery. Chatgidakis (10) studied liver tissue in 157 cases dying of amoebiasis—out of a total autopsy record of 9 500 deaths. In this series, 87 had amoebic liver abscesses and one had acute amoebic hepatitis. The findings in this case were very interesting. Small areas of necrosis were seen and these areas contained remains of necrosed liver cells and a number of lymphocytes.

On the other hand, DaSilva (11) and Donoso *et al.* (12) in a review of a large number of autopsies (5 000 by DaSilva and 10,910 by Donoso *et al.*) which included a number of cases of amoebiasis did not find any evidence of diffuse hepatic lesion due to *E. histolytica* in any case. Kean (13) who is a keen supporter of the school which does not believe in the existence of amoebic hepatitis reviewed the records of 4,478 autopsies at the Board of Health Laboratories, Gorgas Hospital, Ancon. Thirteen of these cases died of amoebiasis. To these he added his own series of 148 cases dying of amoebiasis with clinical evidence of liver involvement. He did not find even one case which could meet the criteria for diagnosis of diffuse amoebic hepatitis. (He has, however, not given the details of what he considers as the criteria for diagnosing this condition).

Liver biopsy studies. According to some, autopsy studies may not be considered as true representatives of the tissue changes as seen in living condition and thus, recently, some liver biopsy studies have been made.

Bonnin and Moretti (14) gave the first report of liver changes in hepatic amoebiasis as seen by liver biopsy. In 4 liver biopsies they found periportal fibrosis and some areas of local inflammatory changes.

Heller *et al.* (15) performed liver biopsies in two cases of amoebic hepatitis. These cases were very interesting in the respect that they were presented as cases of pyrexia of unknown aetiology. Routine investigations were negative and they made the clinical diagnosis of amoebic hepatitis. Liver biopsies showed areas of focal necrosis. Both the cases responded favourably to antiamoebic treatment.

any case. In a repeat biopsy after the treatment they found that there was a

cell pattern

Chaudhuri and Saha (17) performed liver biopsies in 15 cases of active amoebic dysentery and found some non specific inflammatory changes in some of these cases. There was round cell infiltration of the portal tracts and an over-activity of Kupffer cells. No amoebae were found in the lesion.

Similar liver biopsy studies by workers belonging to the other school of

(13) reported biopsy studies in acutely) He did not find evidence non specific) in a single case. In 41 cases, he labelled the tissue as normal and in 6 found evidence of other associated pathology. Three remaining cases had liver abscess.

DaSilva and Torres (18) also in a study of 40 cases of chronic amoebiasis, 7 of which were in acute phase of the disease, did not find any specific change in the liver tissues, which they could correlate with amoebic infection.

Experimental studies. Experimental tests have been employed by a number

amoebic dysentery. In 3 of these cases the functional impairment was considerable. Heilig and Visveswar (21) carried out a study of liver function by Quick's hippuric acid test in 15 cases of amoebic dysentery and 11 cases of acute amoebic hepatitis. They found that prior to therapy the liver functions were much impaired (average value 55.4% of normal). There was marked improvement with emetine therapy. After 6 injections the average value increased to 72.6 of normal and after a full course of 12 injections to 80.1% of normal.

It has been found that in cases of amoebiasis there is a rise in alkaline phosphatase. This has been found in a number of cases of amoebiasis.

Changes in serum proteins have been reported by Gagli and Marinoni (25), Misra and Bajpai (26) and Kasliwal and Sogani (24) in cases of amoebic hepatitis. Powell *et al* (27) have also found similar changes in electrophoretic analysis of serum proteins in amoebic hepatitis though they do not attach much importance to these changes.

The only report which is contrary to these observations is that of Powell *et al* (27) who performed a number of tests of liver function in 49 cases of amoebic dysentery and 31 cases of liver abscess. He found that in all the cases of dysentery where there was no liver abscess, the liver functions were within normal limits and only in the presence of liver abscess, functions showed some derangement.

Experimental studies. Carrera (28) conducted some experiments in kitten. He inoculated 183 kitten per rectum with trophozoites of *E. histolytica*. Two of

He inoculated 183 kitten per rectum with trophozoites of *E. histolytica*. Two of

same lines did not get the same type of results. They injected trophozoites only after the liver was removed. They found that the results were in the same lines.

mesenteric and portal veins. They found amoebae in such lesion (They have not given the details of histological findings of such lesions). Another important observation in this study, which they noted, was that liver abscess could be produced by injection of *E. histolytica* in the portal vein only when there were amoebic lesions in the intestines. In the absence of such intestinal lesions no

specific liver changes?

Councilman and Lafleur (1) commenting upon the histogenesis of the diffuse hepatic changes observed by them in an autopsy in a case of amoebiasis suggested that these changes are due to the absorption of chemical products of amoebae from the intestinal canal.

Rogers (3) expressed the view that amoebae reach in the liver become entangled in blood clots in the intralobular veins producing congestion of the liver. The vast majority of amoebae then undergo degeneration but produce changes in the liver which he termed pre suppurative stage of amoebic hepatitis.

Palmer (8) remarked that in the development of hepatic amoebiasis if an abscess does not form and the process is progressive this terminates in an irregularly scarred liver.

Craig (9) stated that the diffuse hepatic lesions represent the foci of infec-

lytica lying in a zone of completely cytolysed material. This may progress either to true abscess formation or healing occurs with connective tissue replacement.

According to Faust (31) amoebic hepatitis should be considered as due to multiple small colonies of *E. histolytica*, although in most cases the amoebae do not colonize the liver parenchyma but die as the result of amoebostatic action of the liver.

DeBakey and Ochsner (32) and Blanc (33, 34) have suggested that in the early phase of amoebic hepatitis there is balance between two processes: (i) progression to supuration and pus formation and (ii) regression towards healing by scar tissue.

Kasliwal and Bhatia (5) expressed the view that the diffuse hepatic changes are caused by *E. histolytica* and not by bacterial association. They cited in their support the observations of Wahi and Tandon (35) who found similar liver changes in other protozoal infection like malaria and kala-azar.

It is believed that these lesions are due to *E. histolytica* and that these

DaSilva and Torres (18) consider that the mild hepatic lesions which correspond to the designation of amoebic hepatitis are due either to the absorp-

tion of bacterial products through the damaged bowel or to some nutritional deficit co-existent with the parasite infection or caused by it. Thus, Dunlap *et al* (36) conducted liver biopsies in 88 patients suffering from surgical disorders of abdomen (other than amoebiasis) and found that the lesions are non-specific and there is not much difference in the histological changes even in such diverse condition as peptic ulcer, pancreatitis, cholecystitis etc. The lesions described by them resembled those described by other workers (37) in amoebic hepatitis.

We started work on the problem of hepatic amoebiasis in 1954. Part of this work has already been reported (4, 5, 24) and the work is still being continued (38).

(hepatitis in 16% cases, abscess in 2.2% cases)

Seeing such a high incidence of hepatic amoebiasis we started liver biopsy in our patients with intestinal amoebiasis with stool repeatedly positive for *E. histolytica*. A thorough clinical examination was done to find evidence of any associated infection like malaria etc. which might affect the liver. Biopsy was performed in 30 cases with amoebic pathology only. The following histological

in the same zone of hepatic lobule and pyknosis or ballooning of nuclei. Some patches of cells were anuclear. In addition areas of focal necrosis provided another frequent change being present in 21 (70%) cases. This consisted of dissolution of a patch of parenchymal cells which were overrun by a motley of

biopsy, in others, several such wide patches were seen. The trophozoites of endamoeba described by some workers in experimental amoebiasis were looked for and could not be identified with certainty.

Reticuloendotheliosis was present in 28 (93.33%) cases. This also varied

contain a light brown pigment.

Portal infiltration was seen in 12 (40%) cases. The infiltration cells were chiefly mononuclear, although a few polymorphs were also included. This change, however, was never severe.

Fibrosis portal or intralobular was conspicuous by its absence in all cases but one (3.33%). In this case, the fibrosis was limited to the portal area which was considerably enlarged and included 2-3 proliferating biliary ductules. The fibrosis did not extend into the lobule and the normal lobular relationships were still maintained.

consisted of cases with no hepatomegaly or slight nontender hepatomegaly. Group II (16 cases) consisted of cases of frank amoebic abscess. The idea of this classification was to divide cases of intestinal amoebiasis with no clear cut (clinically) involvement of the liver, the second consisting of cases of intestinal amoebiasis with clinical evidence of liver involvement but no demonstrable abscess.

cholinesterase activity, alkaline phosphatases and serum protein fractionation by electrophoresis were also done.

A fall in serum cholinesterase levels was seen in cases of amoebiasis. The fall in cholinesterase activity was consistent with the severity of liver damage. The decrease was only in 27.2% cases of Group I, 56.2% cases of Group II and in 100% cases of Group III.

Alkaline phosphatase levels were normal in all the cases of Group I and Group II but high values were seen in 66.6% cases of Group III.

- (a) Liver enlarged slightly below the costal margin and tender on pressure
- (b) Radiological evidence of hepatomegaly and impairment of movement of right dome of diaphragm
- (c) *I. histolytica* cyst demonstrated in stool
- (d) Response to antiamoebic treatment in the form of disappearance of liver and in general condition

Liver biopsy in these cases showed changes ranging from congestion of portal

infiltration

Liver abscess (4 cases) Liver was enlarged and very tender. Anchovy sauce pus aspirated out and sterile on culture except, in one case where the initial aspiration contained frank yellow thick pus (about 10 cc) and streptococci were cultured from that specimen followed by anchovy sauce pus sterile on culture. *I. histolytica* could not be demonstrated in 2 cases even on repeated examinations and by concentration method.

protein fractionation did show some changes (24). Thus, the lowering of cholinesterase activity in 56.2% of cases of group II (who had no clinically demonstrable liver abscess) indicates that there is some damage of liver cells even in these cases and this may be due to amoebic hepatitis. The findings of Heilig and Visveswar (21) also are in the same line and they have shown that the functional impairment shows improvement after successful antiamoebic treatment.

fibroblasts. The liver cell pattern remains normal except for occasional foci of liver cell necrosis and cellular disorganisation. These changes confirm our previous observations (5). In liver abscess cases in biopsy taken from near the

by scar tissue

With regard to changes in transaminase activity it was seen that in amoebic hepatitis the levels ranged from 6 to 52 units of S G O T (mean value 21.8) and 1.24 units of S G P T (mean value 7.7). In 4 cases of amoebic liver abscess the values of transaminase activities ranged from 20-216 units of S G O T (mean value 100.3) and 1-145 units of S G P T (mean value 50.6).

the other findings of amoebic hepatitis stage, there is also a pre-hepatitis stage or

an early hepatitis where the involvement is normal. This is probably the same stage which Klatzkin (6) has termed as subacute and chronic forms of amoebic hepatitis.

From these observations our conclusions are

- (a) That there is some degree of hepatic involvement in a very large majority of cases of amoebiasis and Radke (11) has correctly remarked that there is hepatic involvement in almost every case of amoebiasis.
- (b) That there are three stages of liver involvement in this disease
 - (i) early hepatitis or prehepatitis stage which is manifested by slight enlargement of liver and minimal or no constitutional disturbances as fever, leucocytosis etc and this stage is the most common of all forms of hepatic amoebiasis and occurs in more than 50% of cases of intestinal amoebiasis.
 - (ii) A definite amoebic hepatitis in which there is hepatomegaly and also constitutional disturbances (it was seen in 16% of cases in our study).
 - (iii) Amoebic liver abscess occurs only in small number of total cases of amoebiasis (2.2% in our study).

This stage of early hepatitis or prehepatitis can be easily recognised if looked for and will very well respond to anti-amoebic treatment. Sodeman (43) has stated that "it is before the stage of acute hepatitis" can be seen.

another clinical picture of amoebiasis with hepatic change.

SUMMARY

A clinical study of the incidence of hepatitis in a large number of cases of

but bacterial associates alone cannot account for such changes specially reticuloendothelial hyperplasia.

It is felt that there is a definite stage of amoebic hepatitis which is a pre-hepatitis.

ACKNOWLEDGMENT

We are thankful to Dr J. C. Sarm, Reader in Pathology, for helping us in the histopathological study of liver biopsies and Shri I. G. Sogani of the Department of Medicine, for his help in carrying out estimation of transaminase activities.

REFERENCES

1. J. N. W. T. and T. A. (1891) *Totus Hottus Hottus Reports* 2: 395
2. "
3. "
4. "
5. "
6. "
7. "
8. "
9. " *us p* 117 Williams
10. "
11. "
12. *Rev Med Jale* 80: 413
13. " *us p* 117 Williams
14. "
15. "
16. " *us p* 117 Williams
17. "
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19. "
20. "
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25. " *us p* 117 Williams
26. "
- 27(a) " *us p* 117 Williams
- 27(b) " *us p* 117 Williams
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29. " *us p* 117 Williams
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DISCUSSION

Dr J. N. W. T. and T. A.

Q. As cellular reaction will be better when the defence reaction is good and not otherwise. I think cellular infiltration in your slides is due to resistance.

Ans. I do not agree with you. In my opinion cellular reaction is entirely due to amoebic infection.

Dr S. H. Zaidi, Lucknow

Q. I think there is no marked cellular infiltration in your slide.

Ans No Cellular reaction is of considerable degree in my slides
Dr V S Mangalik, Lucknow

Q Various workers have reported different kinds of reaction in hepatic amoebiasis like monocytic lymphocytic, reticuloendothelial, fibrocytic reaction. It may appear these are not specific to amoebic infection but may be non specific depending on other coincidental toxins, etc

Ans Cellular infiltration can partly be due to malnutrition but malnutrition cannot be the sole factor

PLEURO PULMONARY AMOEBIASIS

H. N. TANDON AND B. K. KHANNA

From the Postgraduate Medical College, Lucknow

below in detail

CASE REPORTS

I R. S. aged 22 years, clay-pot maker by occupation was admitted to our hospital on 24.3.58 with complaints of low grade fever, cough, moderate hemoptysis (four times) and pain in right side of chest for last one month. The

negative X-ray chest revealed a shadow at the right base suggestive of pneu-

tomography of the right base, failed to demonstrate the ring shadow. Liver receded back.

II N., aged 40 years, was admitted to our hospital on 18.12.58 with complaints of cough, low-grade fever, expectoration and hemoptysis at home for last 15 days. The patient had developed 15 days back high fever with rigor which came down after a day by itself. Thereafter the patient started having excessive dry cough.

about 2 oz. in amount.

blackish red. It was

sputum persisted. On examination, right infrascapular area was found to be dull with diminished air entry. The liver was not palpable. X-ray chest revealed raised diaphragm on the right side with irregular contour. In its middle, there

was a rounded elevation. On screening the diaphragmatic movements appeared to be restricted. Increased reticular markings were visible in right lower zone. Another X ray following a diagnostic pneumoperitoneum revealed a nodular swelling in the contour of the liver. The patient was afebrile and nontoxic. The sputum was found repeatedly negative for *M. tuberculosis*. Tuberculin test with 5 TU PPD was found to be positive. The patient was diagnosed to be a case of amoebic liver abscess which had ruptured through the diaphragm and its contents evacuated out through the bronchi. To that effect, the sputum was examined for vegetative forms of amoeba, but was found to be negative. The patient was given a course of emetine hydrochloride injections as in the previous case. After 10 days, the patient felt much better and cough disappeared. Radiologically, the reticular markings at the right base diminished and the patient

III

in our
and pa
attack of diarrhoea after which he had high fever with rigor which lasted for 6 days. The fever persisted thereafter. He reported to a physician who diagnosed the condition as tuberculous pleurisy and put him on streptomycin 1 g. I.M. O.D. and I.N.H. 200 mg per day. However even after two months of treatment his condition went downhill. He then consulted the senior author (R.N.T.) who diagnosed him to be a case of amoebic liver abscess with right sided empyema (amoebic?). Chloroquine was started at home which brought his temperature down to normal. The patient however continued to complain of severe pain in the right chest and hence was hospitalized. In his statement he revealed a past history of *diabetes mellitus* which was controlled with 2 tablets of tolebutamids per day. The history of amoebic dysentery was also forthcoming. Aspiration of the right pleural cavity brought out anchovy sauce pus which contained vegetative amoeba. The stools and sputum were found negative for amoeba. The patient was put on a course of emetine and chloroquine and repeated aspiration of the chest. His condition improved initially. But all of a sudden on the 30th October 1959 he developed acute cor Pulmonale and died within a span of three hours.

DISCUSSION

In such a short series, as that of ours although one may not be justified in drawing definite conclusions yet certain important inferences can be drawn from our study.

The duration of illness in our series of cases did not extend beyond three months. This observation goes to stress the earlier observations of Chakravarti (+) that the history in these cases is usually short. Important complaints in our series of cases were cough (5 cases) fever (5 cases) pain in right chest (4 cases)

two instances. One of these cases was a diabetic.

Recent history of dysentery was obtainable in 4 cases. In one case inspite of such denial, the incidence of repeated diarrhoea in the past was ascertained. Amoebic cysts in the stools could be obtained in only one instance.

Tuberculin test with 5 TU was found repeatedly negative in 4 cases. This again reaffirms the claim that the presence of negative tuberculin test in hospitalized cases should lead one to suspect non tuberculous conditions.



RS
X-ray chest on admission



N
X-ray chest on admission



RDS
X-ray chest on admission



R S
Following therapy



N
X ray chest following therapy

Sputum was found to be negative for vegetative forms of amoeba in our series of cases. Chakravarti (4) has also had similar experiences.

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tion
whir

amoebic empyema

The third case (R D S) was a diabetic. Not only his condition on admission was moribund, he was fairly dyspnoeic also because of the pus filling the whole of the right hemithorax. After two or three aspirations, it was found impossible

favour

Abdel Hakim and Higar (6) have mentioned the following modes of infection of the intrathoracic viscera

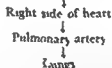
(i) Primary pulmonary amoebiasis

Embolism of amoeba in the bowel reaches inferior vena cava through middle and inferior hemorrhoidal veins. This picture appears independently of hepatic involvement and may simulate broncho-pneumonia or military tuberculosis

(ii) Secondary pulmonary amoebiasis

The pulmonary involvement in these cases is secondary to hepatic pathology. The routes of infection are

- (a) direct extension through the diaphragm
- (b) embolization from thrombosed hepatic vein to inferior vena cava



In our series of cases, the clinical history and the radiological picture point in favour of secondary involvement of the lungs. In 2 cases we have been able to demonstrate clearly the adhesion of liver to the diaphragm. In the other cases also this appears to be a potent possibility.

Therapy with emetine hydrochloride, 1 gr given by I.M.I. daily for 10 days, has been found to produce a satisfactory response in all but one case who was diabetic and was admitted in a moribund condition.

SUMMARY

Five cases of pleuropulmonary amoebiasis admitted to our hospital during the past two years have been recorded. A brief discussion on their clinical feature and response to therapy has been presented.

REFERENCES

- 1 El Mosti, A and Mousa, A G (1951), *Ga. Karr Ann Clin Soc*, Cairo, 17: 1
- 2 Chakravarti, A (1951), *J Indian Med Assoc*, 20: 399
- 3 *Idem* (1952), *ibid*, 21: 387
- 4 *Idem* (1953) *ibid* 22: 418
- 5 Deb, P N (1954), *ibid* 24: 62
- 6 Abdel Hamim, M and H gazi A M (1958), *Dis Chest*, 34: 607

DISCUSSIONS

DR P K CHATTERJEE, Calcutta

Q Is it necessary to do a diagnostic pneumo-peritoneum in a case of suspected liver abscess bursting into the lungs? I think there is definite risk of the abscess bursting into peritoneum

Ans I agree. There is a possibility of abscess bursting into peritoneum. Yet, we did it, to elucidate the fibrous adhesion.

DR P K GHOSH, Calcutta

I concur with Dr Tandon that pulmonary amoebiasis is more commonly an extension from liver. I had 3 cases under me which all were at right base and above liver. But in one of my cases he was treated for 6 months for pulmonary tuberculosis with S M and I N H. Finding no relief, he was admitted under me in hospital for treatment. He coughed up anchovy sauce fluid in sputum which under microscope showed liver cells. It completely cleared up with anti-amoebic treatment. He is well till today—8 years after treatment.

I do not agree that in the case presented with the lungs up, especially (b) the upper lobe cavity.

Ans I do not think that amoebic liver abscess can push lungs up to such a great extent as shown in the X-ray. In the X-ray plate the lung tissue is seen only in the upper zone.

ANTI-AMOEBIIC DRUGS—A REVIEW

JEAN DRUEY

de La Ciba Societe Anonyme, Basle, Switzerland

the bowels, the intestine also becomes sick, rough and ulcerated. It is a long illness, painful and dangerous. If treatment is begun while the body still has

altered to

est was devised by K. M. Lynch (1915) and was more recently (1937) worked out by W. R. Jones in particular. C. Dobell was probably the first (1929) to use monkeys systematically, which were then employed extensively by the school of H. H. Anderson. The cultivation of *Entamoeba histolytica*, *in vitro* presented great difficulties as it flourishes only in the presence of commensals. The first usable

Chemistry was well equipped to make available its large arsenal whenever new biological test methods made their appearance. This process began in the 1920's and continues at a lively rate to day.

India may be credited with having discovered for the first time active principles in plants at a very early date which are therapeutically useful even measured by the latest research, from the plant called *Lurchi* which contains conessine as its principal alkaloid.

The first anti dysenteric drug to enjoy great popularity was *Ipecacuanha* from South America whose root contains emetine as its main alkaloid. It was first mentioned in a travel book by Samuel Purchas that appeared in 1625. From it Pelletier isolated emetine in 1817.

The various groups of anti amoebics

accessible to a direct comparison although even here striking differences are often manifest depending on method. The chief remaining uncertainty in some cases is whether the effect of amoebicidal action or rather of culture associates. For the strength of the amoeba

is still more difficult in animal experiments since as yet there is no absolutely satisfactory way of infecting mammals experimentally and particularly because the various species do not produce results that can be compared. Above all the therapeutic spectrum in the different animal tests is subject to quite considerable fluctuations. Another difficulty in comparative evaluation is the fact that *in vitro* and *in vivo* experiments often diverge greatly so that high activity *in vitro* is of small practical significance. On the other hand one may take heart from the fact that almost all anti amoebic preparations which have proved of

the same laboratory

This review includes the following groups of drugs

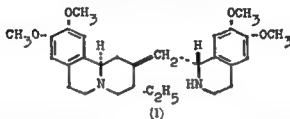
- A Natural products
 - Ipecacuanha*
 - Holarrhena*
 - Simaruba glauca*
 - Brucea* and other plant principles
 - Antibiotics
- B Synthetic products
 - Emetine analogues long chain diamines
 - Quinoline and acridine derivatives
 - Arsenicals
 - Chloracetamides
 - Miscellaneous structures
 - Phenanthroline derivatives

Special emphasis will be given to the last group of phenanthrolines developed in the last ten years in our laboratories

A Natural products

(a) *Ipecacuanha* group

Emetine (I) is still widely used for the hepatic form of amoebiasis, although Anderson, in a review article in 1950, stated 'that its toxicity precludes its recommendation at present'. Its usefulness is seriously limited by its cumulative properties its cardiotoxic effects and its narrow margin of safety. Emetine served as a standard for comparison of new drugs, and its chemical structure served as a model for the development of similar compounds



A new total synthesis of natural emetine in its true stereo chemical configuration was described by O. Schneider and Collaborators of Hoffmann-La Roche in Basle. There is no doubt that emetine belongs to the true amoebicidal drugs i.e. to those which act directly on the amoeba and not by killing the symbionts. For this reason it was and still is useful in hepatic abscess, where there are no

Neipp, 1958)

was found to be 5 mg/kg during 5 days initiated the rat test, worked with doses therapy. In his monkey experiments,

was even more active than emetine *in vitro* and in the monkey

(b) *Holarrhena*

This plant is already mentioned as the oldest of the amoebicidal drugs. In Sanskrit it was called 'Indrayava' or 'Vatsaka' but it became especially known as 'kuichi'. Conessine is the main alkaloid. The biological action of conessine was extensively studied by Chopra in 1927 in a classical paper. Favourable clinical results were published by Acton and Chopra in 1933. Later on French scientists became especially interested in this drug. It has a lower activity than emetine and is reported to be toxic.

(c) *Simaruba glauca*

A comprehensive review on glaucarubin has been given by A. C. Cuckler in 1958. Chemically this compound was described by F. A. Ham *et al.* as having

* J. Amer. Chem. Soc., 1951, 73: 6066

the formula $C_{25}H_{38}O_{10}$, containing a lactone grouping, an ester group and 6 hydroxyl groups. *In vitro*, it proved to be about 2 to 11 times less active than emetine, using the same testing procedure. Its action on amoebae is direct. The curative dose in rats according to Cuckler, is 25–50 mg/kg/day. Glau-carubin is also active in guinea-pigs, where it proved to be one of the best amoebicidal drugs. For dogs, it is rather toxic but the doses for curative effects in colitis are also much lower. The therapeutic dosage for man was found to be 3.5 mg/kg/day for ten days, i.e. a total dose of 2 to 3 g.

(d) *Brucea* and other plant principles

is claimed to have amoebicidal properties *in vitro* and *in vivo* on the rat (M S Kiang *et al*, see *Chem Abstr*, 53: 11646).

(e) *Antibiotics*

Many antibiotics have been reported as having anti amoebic properties

TABLE I
In vitro amoebicidal activity of antibiotics*

Group I, amoebicidal	at 5 γ /cc
Actidione	Fumagillin
Anisomycin	Prodigiosin
Group II, amoebicidal	at 63 to 300 γ /cc
Chlortetracycline	Purromycin
Oxytetracycline	
Group III, amoebicidal	at 500 to 1000 γ /cc
Azaserine	Streptothricin
Carbomycin	Tetracycline
Chloramphenicol	
Group IV, amoebicidal	at 1000 to 2000 γ /cc
Bactracin	Vindogrusin
Neomycin (?)	
Group V, not amoebicidal	at 2000 γ /cc
Streptomycin	Penicillin G
Dihydrostreptomycin	Antibiotic PA 105
Erythromycin	

* According to Thompson *et al* (1956), *Antibiotics & Chemotherapy* 6: 346

TABLE II

*Comparison of antibiotics on the basis of retal *in vitro* potency in laboratory tests and over-all appraisal as potential drugs for intestinal amoebiasis**

Drug	Relative ratings†			Potential usefulness in human intestinal amoebiasis
	Activity <i>in vitro</i>	Activity in rats	Activity in dogs	
Actidione	I	I	I	C
Axaserine	III	V		C
Anisomycin	I	II		A or B
Bacitracin	IV		IV	B
Carbomycin	III	IV	IV	B
Chloramphenicol	III	V	IV	B
Chlortetracycline	II	III	III	A
Dihydrostreptomycin	V	V		B
Erythromycin stearate		III		A or B
Fumagillin	I	I	I	A
Neomycin	IV	IV		B
Oxytetracycline	II	II	III	A
PA 103	V	III	IV	B
Penicillin G	V	IV	IV	B
Prodigiosin	I	II		C
Puromycin	II	II	II	A or B
Streptomycin	V	V		B
Streptothricin	III	II	II	C
Tetracycline	III	IV	IV	B
Vidodigraisin	IV	III	III	A or B

* According to Thompson *et al* (1956), *Antibiotics & Chemotherapy*, 6: 346

† I through V represents order of decreasing potency. A, potentially adequate alone, B, probably useful mainly as adjuncts to more specific antiamoebic drugs, C, essentially ruled out by toxicity

Five groups and four groups, respectively, were also formed according to activity in rats and in dogs. An overall appraisal results therefrom, as seen in Table II. Interesting compounds like actidione, prodigiosin and streptothricin were ruled out, especially because of their toxicity. Others, marked with an "A" in the table, like fumagillin, proved to be clinically very useful, but toxic side reactions have since hindered their general use in humans. The same is true for the high-ranging puromycin. Terramycin and aureomycin remain for treatment of amoebiasis. Experimentally, rather high doses of aureomycin—100 mg/kg/day—are needed to cure rats in our experiments, i.e. higher doses than of simple synthetic drugs like phenanthroline-quinone, to which we shall refer later.

Another antibiotic might be added to the table of Thompson, namely spiramycin, which was discovered independently in our laboratories and by

the formula $C_{25}H_{36}O_{10}$ containing a lactone grouping an ester group and 6 hydroxyl groups. *In vitro*, it proved to be about 2 to 8 times less active than emetine, using the same testing procedure. Its action on amoebae is direct is 25–50 mg/kg/day. Glauved to be one of the best amoebicidal doses for curative effects in colitis are also much lower. The therapeutic dosage for man was found to be 3.5 mg/kg/day for ten days, i.e. a total dose of 2 to 3 g.

(d) *Brucea* and other plant principles

Several other plants have been reported as containing anti amoebic principles. The *Brucea sumatrana* tree has to be especially mentioned, the fruits of which are used. It contains the Chinese anti amoebic Kosam or Ya tan tzu, the active principle seems to be a glycoside called yatanside. A white amorphous compound with glycosidal properties was described by C. Y. Sung in 1949 ("brucealin"). An *in vitro* activity somewhat less than that of emetine is reported. Other plants of interest may be the *Stephania rotunda* and *S. hernandifolia*, the roots of which are used in India against dysenteric illnesses.

In a recent paper by Chinese authors, a root extract of *Anemone chinensis* is claimed to have amoebicidal properties *in vitro* and *in vivo* on the rat (M. S. Kiang *et al.*, see *Chem. Abstr.*, 53: 11646).

(e) Antibiotics

Many antibiotics have been reported as having anti amoebic properties

on the first in 1956. They divided a large number of antibiotics into several *in vitro* as well as in animal tests (Table I). This last animal for the evaluation of drugs

TABLE I

*In vitro amoebicidal activity of antibiotics**

Group I amoebicidal	at 5 γ/cc
Actidione	Fumagillin
Anisomycin	Prodiosin
Group II amoebicidal	at 63 to 300 γ/cc
Chlortetracycline	Puromycin
Oxytetracycline	
Group III, amoebicidal	at 500 to 1000 γ/cc
Azaserine	Streptothricin
Carbomycin	Tetracycline
Chloramphenicol	
Group IV, amoebicidal	at 1000 to 2000 γ/cc
Bacitracin	Vindogracin
Neomycin (?)	
Group V, not amoebicidal	at 2000 γ/cc
Streptomycin	Penicillin G
Dihydrostreptomycin	Antibiotic PA 105
Erythromycin	

* According to Thompson *et al.* (1956), *Antibiotics & Chemotherapy*, 6: 346

TABLE II

*Comparison of antibiotics on the basis of relative potency in laboratory tests and over all appraisal as potential drugs for intestinal amoebiasis**

Drug	Relative ratings†			Potential usefulness in human intestinal amoebiasis
	Activity <i>in vitro</i>	Activity in rats	Activity in dogs	
Actidione	I	I	I	C
Azaaserine	III	V		C
Anisomycin	I	II		A or B
Bacitracin	IV		IV	B
Carbomycin	III	IV	IV	B
Chloramphenicol	III	V	IV	B
Chlortetracycline	II	III	III	A
Dihydrostreptomycin	V	V		B
Erythromycin stearate		III		A or B
Fumagillin	I	I	I	A
Neomycin	IV	IV		B
Oxytetracycline	II	II	III	A
PA 105	V	III	IV	B
Penicillin G	V	IV	IV	B
Prodigiosin	I	II		C
Puromycin	II	II	II	A or B
Streptomycin	V	V		B
Streptothricin	III	II	II	B
Tetracycline	III	IV	IV	B
Vaidogrisein	IV	III	III	A or B

* According to Thompson *et al.* (1956) *Antibiotics C. Chemotherapy*, 6: 346

† I through V represents order of decreasing potency. A, potentially adequate alone; B, probably useful mainly as adjuncts to more specific antiamoebic drugs; C, essentially ruled out by toxicity.

Five groups and four groups, respectively, were also formed according to activity in rats and in dogs. An analysis of these results is given in Table II. Interesting

were ruled out especially

"A" in the table, like

side-reactions have since hindered their general use in humans. The same is true for the high ranging puromycin. Terramycin and aureomycin remain for treatment of amoebiasis. Experimentally, rather high doses of aureomycin—100 mg/kg/day—are needed to cure rats in our experiments, i.e. higher doses than of simple synthetic drugs like phenanthroline quinone, to which we shall refer later.

Another antibiotic might be added to the table of Thompson, namely spiramycin, which was discovered independently in our laboratories and by Rhone-Poulenc. In our hands it proved to be active in rats at a dose as low as 10 mg/kg/day. In man, too, it seems to be quite effective.

The extremely high potency *in vitro* of compounds like fumagillin and actidione, displaying amoebicidal activity in concentrations of less than 0.1 mg

per liter or approaching a dilution of 1 : 100,000 000 is worthy of special attention. Actidione is the most active compound tried in rats in our laboratory. The minimal effective dose was as low as 0.5 mg/kg/day, while for fumagillin this figure was about 4 times higher.

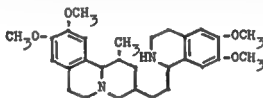
B : Synthetic products

(a) Emetine analogues Various diamine type structures

Ever since the early days when chemical synthesis was applied to the search for new drugs, compounds closely similar to biologically active natural products as well as compounds resulting at random from the chemist's work have been screened for drug properties.

In the search for anti amoebic drugs too we encounter both types of approach. The purely synthetic product not related to any natural product, was developed first, i.e. chiniofon or *jaltren* which will shortly be discussed.

The first experiments with synthetic emetine relatives go back to the late twenties when a tentative structural formula for emetine became known and when screening possibilities for anti amoebic activity were available. Pyman in 1927 attributed the following formula (II) to emetine.

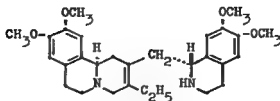


Emetine according to Brindley & Pyman, Soc. 1927, 1069

(II)

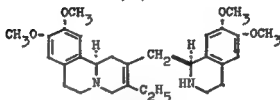
Pyman prepared *inter alia* a compound composed of two dimethoxytetrahydroquinoline groups linked by a 5 carbon chain in 1-1-position, a logical consequence of his emetine formula. It was devoid of anti amoebic activity, as were the analogues with a linking chain of 4 and of 8 carbons. According to the correct emetine formula there should have been 3 carbons only. Compounds of this type were prepared later, in 1952 by Fulton, but even a product lacking only one carbon atom of emetine was only slightly active (*in vitro*). How specific the structure of emetine is has recently been shown by our colleagues from the Roche laboratories in Basle. The racemic 2 dehydro emetine (III) proved microscopically inactive. The stereochemistry has not yet been synthesized and tested.

The length of linking carbon chains was varied in Goodson (1948) and in Osbond (1951). Osbond (1959) is a de ethylemetine, it is inactive. Some representatives of the type of Goodson (1948) were claimed to be active in rats. The diamine type of Goodson 1948 (secondary amines) and Hall 1952 (primary amines) are already far away from emetine, the aromatic rings having become simple alkyl chains. Already in 1937 Pyman claimed amoebicidal properties for the simple tertiary diamine.



rac. 2-dehydro-emetine
highly active

(III)



rac. 2-dehydro-iso-emetine
inactive

(IV)

Dhar and Mahboob prepared aliphatically substituted diamines and these were tested *in vitro* by Kaushiva. The most active compounds were found with a central chain of 9 carbon atoms with morpholine or piperidine as a substituent on one side and a hexyl or dodecylamine, the N being secondary, on the other side.

Coming back to products having a closer relationship to natural products, I have to refer to the interesting quinine relatives presented here in Lucknow at last year's Symposium on Chemotherapy by Popli, Dhar and Bhandari (Chart 2). It is interesting to note, that desoxyhexahydroquinine, in which the two nitrogen atoms have about the same distance as in emetine is active in intestinal amoebiasis. However, the stereochemical isomer of the quinidine series was inactive. The second compound has also been described as possessing *in vivo* activity.

(b) Quinoline and acridine derivatives

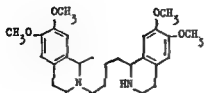
To this group belong compounds of amoebiasis, the also well-known as chinchona properties was quite

man
know

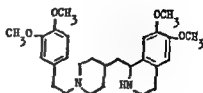
immediately the origin of these iodine-containing compounds lies in the disinfectant virtues of iodine itself, and it was hoped to achieve improved properties by incorporating the iodine in organic molecules. It is not surprising, therefore, that the di-iodo-analogue of vioform, duodoquin, had a halo of efficaciousness when it was recommended for the treatment of amoebiasis in 1936 by A. C. Tenney.

The three drugs are shown in (Chart 3). Clinically they are useful in intestinal amoebiasis, but not in the hepatic form.

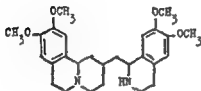
Chart 1



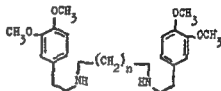
J.M. Osbond 1951



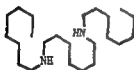
J.M. Osbond 1959



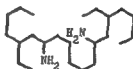
J.M. Osbond 1959



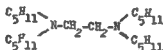
J.A. Goodson 1948



J.A. Goodson 1948

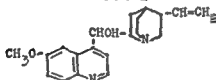


D.M. Hall 1952



F.L. Pyman 1937

Chart 2



Quinine

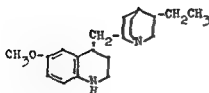
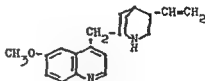
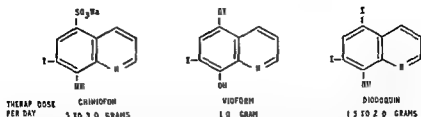
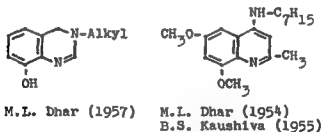
9-Desoxy-hexahydro-quinine
Popli et al. (1958) α -[3-Vinyl-piperidyl-(4)]- γ -[6-Methoxy-quinolyl-(4')]-
propane
Popli et al. (1958)

Chart 3



The halogenated hydroxyquinolines being the first synthetic anti amoebic drug, it is somewhat surprising that they were not followed by a large series of congeners with similar or better therapeutic properties. Several hydroxyquinolines have been tried they were tabulated by Thompson (*Amer J Trop Med Hyg*, 1955 4: 242). Data on analogues of other heterocyclic derivatives are very rare. At this Institute this problem with 8 hydroxy quina-zoline derivatives was considered (Chart 4). They do not seem to have shown outstanding properties.

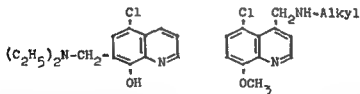
Chart 4



The 6, 8 dimethoxy quinaldine derivatives should be mentioned here too,

uqu 4

Chart 5



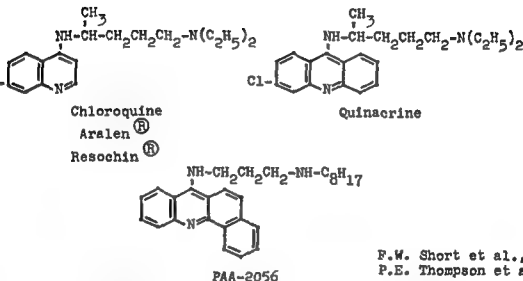
J.H. Burckhalter (1951)
P.E. Thompson (1955)

R. Gopalchari (1957)

The *in vitro* activity compares favourably with emetine. *In vivo*, however, rather high doses are needed to cure intestinal amoebiasis in rats. I am not aware of as to whether clinical results have been published. Dozens of analogues were prepared and tried in animals. In the hamster the compound is probably

too rapidly metabolized to be active systemically. In the Chart 5 another

Chart 6



In vitro amoebicidal conc. 2.5-20 µg/cc. Rats 150-200 mg/kg/day for 90-100 p.c. cure
 Dogs 10-20 mg/kg/day for 2 weeks. Hamsters 12.5-50 mg/kg/day for 4 days

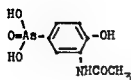
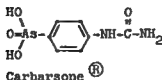
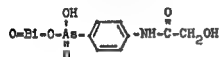
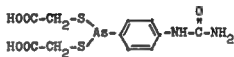
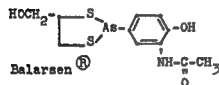
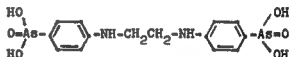
Quinaquine too belongs to this group. From a very large number of similar compounds prepared and tested at the laboratories of Parke Davis & Co. in the U.S. the one called PAA 2056 seemed to be the most interesting. *In vitro*, PAA 2056 is almost as potent as emetine (depending on the test conditions). It has a direct effect on the amoebae. It is moderately effective against symptomatic intestinal amoebic dysentery in dogs and roughly 11 times as active as chloroquine against hepatic amoebiasis in hamsters. It looks promising.

(c) Arsenicals

The chloroquine type of compounds brought us back chemically to the diamine type structure. They were developed however, by following a completely different way than was taken with the emetine analogues.

The starting material, 4-oxo-1,2-diphenyl-3-methyl-5-pyrazolone, was prepared in 1923. The compound, which is shown in Chart 7, is probably still the most common of its triazole obtained. By reaction back to a hypothesis of Voegelin in 1925 that a diamine reacts with reduced

Chart 7

Acetarsone (Stovarsol[®])Carbarsone[®]Glycobiarsone (Milibis[®])Thiocarbarsone[®]Balarsen[®]

Diphetarzone

glutathione. The first thioarsenites were prepared in the early thirties, but especially H. H. Anderson followed up this idea further after World War II (1947).

Milibis (R), generic name glycobiarsone, is a mixed arsenical bismuth-organic compound, found in Germany in 1943 (Hauer). It is not resorbed from the intestine and is used mainly in combination with chloroquine.

In our hands carbarsone (R) and especially milibis (R) were weak amoebicidal drugs. Comparative activity of arsenicals and other amoebicidal drugs is shown in Chart 8.

Chart 8

	In rats min. eff. dose mg/kg/day	in humans g per day
Emetine	5	0.06
Fumagillin	2	0.01 to 0.05
Aureomycin (R)	100	2.0
Chloroquine	100	0.9 to 0.3
Carbarsone (R)	250	0.75
Milibis (R)	1000	1.5
Entobex (R)	50	0.3
Vioform (R)	100	1.0

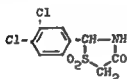
The trivalent arsenic derivatives in this series too were more effective than the pentavalent.

(d) *Haloacetamides*

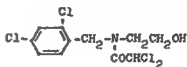
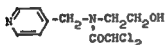
This group (Chart 9) merits being discussed separately as another class of chemicals which went to clinical application from the laboratory desk of a chemist. It is a typical example of fortuitous detection of biological activity in a certain compound out of thousands screened every year. The starting point of this series of chloroacetamides was the observation that a dichlorophenylthiazolone derivative showed some activity in naturally infected hamsters (*Endamoeba criceti*). In pursuing this observation, many similar compounds were prepared and

related to camoquine, chloroquine and to quinacrine. The simple pyridine derivative shown are proved to be the most effective of this series. It is also active against amoebic dysentery in dogs but ineffective against amoebic hepatitis in hamsters. The Logemann variation with *p* nitrophenoxy is claimed to be active in man.

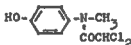
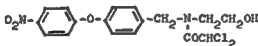
Chart 9



Surrey, 1954

Win 5047 Mantomide[®]

Eislager, 1956

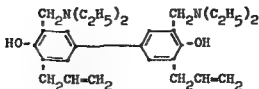
Entamide[®]

Logemann, 1958

(e) *Miscellaneous products*

This heading does not refer to catch all group. It exhibits a drug with

properties were published by Thompson in 1955. Its activity in rats is not outstanding. In our hands, too, it was about three times less effective in the rat-test than known drugs such as vioform or aureomycin. However, many of the



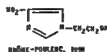
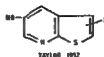
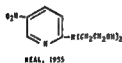
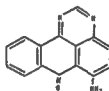
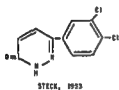
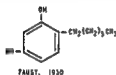
Biallylamicol, PAA 701, SN 6771

Camoform[®]

V

antiamoebic drugs in clinical use have shown that, on a weight basis, from 2 to 70 times as much drug is required for rats as is used in man. The clinical dose of camoform(R) is 1 to 15 g a day for at least five days. On a weight

Chart 10



base it is therefore some little effort at synthesis is required.

com
publ
have summari

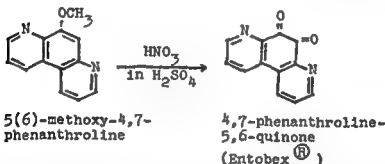
in animals for amoebicidal properties. In Chart 10 a few structures which have already emerged from the chemical side and claimed to have *in vivo* activity, are given.

(f) Entobex

A member of this new group, 4,7-phenanthrolinequinone, became known in this country through several clinical publications.

Chemically, the phenanthroline derivatives, with which we started with the aim of finding new therapeutic potentialities are based on our experience with the 8-hydroxyquinolines and especially visform(R). The hydroxy-quinolines are synthesized by the so-called Skraup process starting with nitro- or aminophenols. With diamino as well as with nitro-aminophenols one gets, by a

Chart 11



two fold Skraup reaction the phenanthroline derivatives either directly or one can proceed step by step, obtaining first an amino hydroxy quinoline derivative and by a second Skraup reaction phenanthroline. The methoxy 4,7-phenanthroline

anti amoebic drugs in common use has been discussed already. Summing up the biological characteristics as they have been determined by Kradolfer and Neipp, the following may be of interest:

The anti amoebic activity is at least equal or superior to that of emetine.

3 In rats, using the Jones procedure, the minimal effective dose (100% cured) was 50 mg/kg. The ratio of effective to toxic dose is 1:110, i.e. 30 times greater than with emetine.

4 The phenanthroline-quinone is excreted in urine and in the bile in an amoebicidal form. Semiquantitative chemical analysis seems to indicate that the










plays a contrary effect, i.e. a bacteriostatic action on colon bacilli.

I should like now to give a summary of structure activity relationships in the large number of phenanthroline quinones investigated biologically in our laboratories.

In Chart 12, I should like to draw your attention to the three isomeric phenanthrolines, the *o*-, *m*- and *p*- or 10-, 7- and 4-phenanthrolines.

Chart 12

Mono-substituted and unsubstituted phenanthrolines






	in vitro	in vivo		in vitro	in vivo
	+	-		+	-
	++	+		+	II
	±	-		±	-
	+	0		±	-
	+				

Without further substituent all the three are almost devoid of antiamoebic properties. The only compound showing some activity in the mono substituted group is the hydroxy-4-7-phenanthroline.

Among the quinones (Chart 13), the one of the 4-7-series proved to be the most active compound, called

Chart 13

Phenanthroline quinones

	in vitro	in vivo
	++	++
	+	+(+)
	+	+
	++	++
	++	++

It is an interesting fact that the hydrogenated effectiveness we investigated in the literature advantages over

the free quinone

Cyclic derivatives were also broadly investigated. I have selected some of them in Chart 15

By cyclization with ethylene diamine, three types of compounds are obtained, the dihydro- or tetrahydro-derivatives or the fully aromatised derivative as a result of some oxido reduction process. None of the cyclic derivatives showed outstanding properties so far as the anti-amoebic activity is concerned. Some compounds proved to be of interest as anti-tumour agents.

Chart 14
Derivatives of 4,7-phenanthroline quinone

	in vitro	in vivo		in vitro	in vivo
	+	+		0	+
	+	0		+	
	++	+(+)		+	+

Chart 15
Cyclic derivatives of phenanthroline quinones

	in vitro	in vivo		in vitro	in vivo
	+	-		0	0
	+	+		+	0
	+			++	+
	0	-			

ENTAMIDE

P T MAIN N W BRISTOW P OXLEY, T I WATKINS G A H WILLIAMS
E C WILMSHURST AND G WOOLFE

From the Medical and Research Departments Boots Pure Drug Co Ltd, Nottingham England

METHODS

The methods used in our research laboratories to test new amoebicidal drugs have been described by Woolfe (1956). Large numbers of chemical compounds are screened and, if activity is noted in any compound, then modification of the molecule is undertaken in order to increase activity and decrease toxicity.

In vitro tests

Drugs which have proved to be active clinically must be active in such a test. We use a microtube method in which serial tenfold dilutions of the drug under test are incubated in capillary tubes with strain M of *Entamoeba histolytica* in an all-liquid medium (liver extract marmite, and horse serum in phosphate buffer at pH 7.2) with rice starch. The tubes are completely filled with liquid, plugged with plasticine, and incubated in a horizontal position so that multipli-

M. T. M. C. A.

TABLE I

Amoebicidal activity—in vitro microtube method

Drug	At concentrations of	
	Inactive	1 in 1,000
Penicillin	Inactive	1 in 1,000
Streptomycin	Inactive	1 in 1,000
Chlortetracycline	Active	1 in 10,000
Oxytetracycline	Active	1 in 10,000
Iodo 8 hydroxyquinoline	Active	1 in 10,000
Emetine hydrochloride	Active	1 in 500,000
Pyrimidine	Active	1 in 10,000,000
Fumagillin	Active	1 in 100,000,000

It would appear that an antibiotic such as fumagillin has direct effect on the amoebae, whereas other antibiotics have little or no direct amoebicidal action. It must be emphasized that this *in vitro* test only indicates which compounds require further study and the ratios of activity for compounds may be quite different in an *in vivo* test.

In vivo tests

Newly weaned rats are used. They are inoculated intracaecally with trophozoites of *E. histolytica* dosed daily for five days with the drug under trial and the state of the caecum of each rat assessed on an arbitrary scale. The average degree of infection (ADI) for each group can be calculated from the following formula: $\frac{\text{ADI}}{\text{ADI of control}} = \text{relative activity}$. We which cative"

They are, however, have a poor dose response curve and high doses are required to completely clear the rats of infection.

RESULTS

Using the methods described, a large number of synthetic compounds of various types were screened. It was found that a series of substituted acetanilides

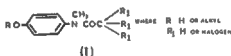
TABLE II

Amoebicidal activity—in vivo (rat test)

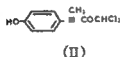
Drug	Just active dose (mg/kg daily for 5 days)
Carbarzone	250
Iodochlorhydroxyquinoline	150
Entamide	10
Fumagilin	3
Chlortetracycline	2.5
Oxytetracycline	1.0

had considerable activity *in vitro* and some were active *in vivo* in the rat. The

A preliminary note on entamide was published by Bristow and others (2)



ENTAMIDE (D. OLAN DE)



Toxicity studies in animals

In view of the high activity of the compound extensive toxicity tests were put in hand by our colleague, Dr M. R. Gurd. The acute LD 50 of entamide orally to mice was about 5.5 g per kg body weight. Rats were dosed daily for several weeks with different dosage levels of entamide and apart from some growth reduction and slight liver and kidney enlargement in 1 g/kg daily, there were no toxic effects. In cats, apart from occasional vomiting at 1 g/kg daily, the compound appeared to be completely non toxic.

Human toxicity studies

to
or
re-

Clinical trials of entamide

Our laboratory studies demonstrated that an — 3
considerable activity
volunteers had reassu
should be submitted to clinical trial

The first pilot trial was undertaken at the Hospital for Tropical Diseases in London by Professor A. W. Woodruff. This unit is well suited for carrying out this type of trial not only because it is particularly interested in amoebiasis but also because the follow up of patients is relatively easy and the problem of reinfection does not occur.

Woodruff, Bell and Schofield (3) have published the results of this trial. They started their trial using a very low dosage of 2 mg. per kg. body weight daily for 10 days for the first 100 patients and then increased the dosage to 12 mg. per kg. body weight daily for the next 100 patients.

treated with 12 mg. or more per kg. body weight daily—which gives a relapse rate of 11.8%. Entamide was found to be most effective in those cases with *F. histolytica* (large stage) cysts in the stools—only one case relapsed out of 22 treated (4.5% relapse rate).

On the basis of this trial the drug was recommended for further trial as a therapeutic agent for chronic cyst passing patients. We have not recommended its use alone in acute dysenteric amoebiasis.

A further trial was reported on by Foll (4) in employees of the Burma Oil Company and their dependants. Over 30% of all prospective employees were found to have amoebiasis and the vegetative form was the commonest type of *E. histolytica* in the stools. Foll used a higher dosage of entamide than Woodruff and his colleagues and his dosage ranged from 20–56 mg. per kg. body weight daily for 10 days. Some were treated as in patients and some as out patients. Fifty-five patients were treated (49 with vegetative forms and 6 with cysts). The relapse and/or reinfection rates of in patients and out patients were as follows:

	In patients	Out patients
At 1 month	11.7%	24.3%
At 2 months	23.2%	40.5%
At 3 months	41.2%	64.9%

By comparing a series of patients treated with emetine bismuth iodide in Burma with a similar series treated in London by Woodruff and his colleagues (3), Foll

have reported on their trials of
ruff and his colleagues (3) that
by about the 5th day of treat

They employed three dosage
veight daily for 10 days. Nine
wed up and in only 1 case was

there ■ recurrence, and this was 6 months after treatment and the cysts promptly

disappeared on 40 mg per kg daily. Five out of 6 cases on 20 mg per kg daily were followed up, and 1 relapsed at 1 month and was retreated success

entamide alone

Apart from the published trials which we have mentioned above, entamide has been the subject of trials in the U.S.A., Kenya, Sudan, the Persian Gulf, South Africa, Malaya, Honduras, and Argentina, and in a few other centres in India. As some of the clinicians concerned either have papers in the press or do intend to publish in the future, we will not mention individual trials, but we can say that in 663 well documented cases which have been treated with entamide the percentage cure-rate at the present time in non acute amoebiasis is 72.2. The following tables indicate results in non acute amoebiasis and in acute amoebic dysentery.

TABLE III
Entamide trials—non acute patients

Trial	No treated	Cured (%) ^a
Woodruff, Bell and Schofield (1956)	45	75% (88% of those given 12 mg/kg/day or more)
Sanjay and Thiruvengadam (1958)	30	87%
Toll (1957)	55	42.6% (54.6% if reinfection rate is 12%)
Toll and Game (1959)	24 53	43.5% 31.6% In patients Out patients
Unpublished trials	436	75.8% (12 centres)
Total	663	72.2%

^a The % cured is expressed as a % of those followed up and not of those treated

TABLE IV
Entamide trials—acute dysenteric patients

Trial	No treated	Cured (%) ^a
Sanjay and Thiruvengadam (1958)	33	51.5%
Other trials	18	66%
Total	51	57%

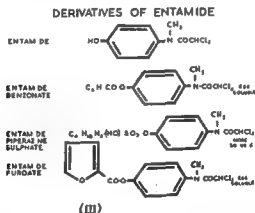
^a The % cured is expressed as a % of those followed up and not of those treated

Absorption and excretion

When the drug was administered to rats, the absorption was estimated by using material tagged with ^{14}C . We found that in rats less than 20% of the drug given appeared in the faeces, most was excreted in the urine. The drug was in fact absorbed very rapidly from the stomach as well as from the intestine, but there was no accumulation of radioactivity in any organ or tissue. Kidney levels were high for a few hours only after dosing, and liver levels were always low.

Derivatives of entamide

The rather disappointing clinical effect in acute amoebiasis made us look further at the series. We were not sure whether the lack of clinical activity was due to too low concentration of entamide in the lumen of the gut because of absorption higher up in the gastro intestinal tract or whether the blood levels were too low to bring an effective concentration of entamide to the amoebae in the ulcers. Accordingly, we prepared derivatives of entamide with both high solubility (and the probability of rapid absorption) and even lower solubility than entamide (to produce a higher concentration in the lower bowel).



These derivatives were tested in the laboratory and two were chosen at this stage for clinical trial. One, entamide piperazine sulphate, was readily soluble in water. The other, entamide benzoate, was considerably less soluble than entamide itself. Both derivatives were highly active in the laboratory tests. The piperazine sulphate was about as active as entamide itself and the benzoate rather more active. Both were of low toxicity and therefore suitable for clinical trial. We also decided to try entamide in the form of enteric coated tablets.

Clinical trials of entamide benzoate and entamide piperazine sulphate

Foll and Game (6) have published some results of trials of these derivatives in Burma, and their paper also reports on an extension of the number of cases treated with entamide itself (Table V).

TABLE V

Drug	In patient or Out patient	No treated	Percentage relapse or reinfection		
			1 month	2 months	3 months
Entamide	IP	24	21.7	47.8	56.5
20-56 mg/kg/day	OP	51	28.8	48.1	65.4
Entamide benzoate	IP	30	22.2	37.9	50.0
30-50 mg/kg/day					
Entamide benzoate	OP	11	33.3	40.0	70.0
50 mg/kg/day					
Entamide p. perazine sulphate	IP	30	23.3	36.7	36.7
50 mg/kg/day	OP	5	20.0	40.0	40.0
E B I					
3 gr. three times daily	IP	12	16.7	33.3	33.3

Foll, and Game, (6)

These results again show a much higher relapse rate for entamide than do those of other workers but it can be seen that the relapse rate with entamide p.

th
ar
effective than the parent drug. Preliminary results from other workers indicate that enteric coating does not improve the efficiency of entamide.

Entamide furoate

While these trials were in progress experimental work continued with the main emphasis on esters of entamide. From the many esters made, the furoate was outstanding in its activity, being several times more active than entamide in rats. Toxicity studies showed that it was even less toxic than entamide and the therapeutic ratio therefore, even greater.

It is too early to give any detailed clinical results, but Woodruff (7), in his recent review article on amoebicides, has indicated that preliminary trials in man are very promising. More clinical investigation has still to be carried out on this derivative.

DISCUSSION

Many vague syndromes and cases of alimentary neurosis have been labelled as amoebiasis, and Sir George McRobert (8) recently referred to this condition as 'chronic remunerative amoebiasis'. Nevertheless true amoebic infection is still a major problem in the world. It seems to us that a cheap, effective, non-

REFERENCES

1. Woolfe, G (1957), *Trans Roy Soc Trop Med*, 51: 320
2. Bristow, N W, Oxley, P. Williams, G A H and Woolfe, G (1936), *Trans Roy Soc Trop Med*, 50: 182.
3. Woodruff, A W, Bell, S and Schofield, F. D (1956), *Trans Roy Soc Trop Med*, 50: 114
4. Fall C V (1957) *J Trop Med Hyg*, 60: 115
5. " " " " " "
6. " " " " " "
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**PAROMOMYCIN AS A THERAPEUTIC SUBSTANCE FOR INTESTINAL
AMOEBIASIS AND BACTERIAL ENTERITIS**

**KENNETH O. COURTNEY, PAUL E. THOMPSON, ROBERT HODGKINSON
AND J. R. FITZSIMMONS**

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Read by Dr. B. S. Kaushik, Central Drug Research Institute, Lucknow*

Paromomycin (Humatin) is the Parke, Davis & Company trademark for

concise summary of the important features of paromomycin observed in the laboratory and to indicate the present status of the drug relative to the chemotherapy of certain of the enteric diseases.

LABORATORY STUDIES

Microbiologic properties in vitro and in experimental animals Paromomycin is highly active *in vitro* against many types of both gram-negative and gram-positive bacteria (2, 11, 13). It also proved to be very effective when tested parenterally against systemic bacterial infections in mice (1, 4, 13).

This antibiotic also has very high activity against *Entamoeba histolytica* (3).

normal bacteria

More importantly, the drug is effective against amoebae *in vivo*: The antibiotic, when fed in the diet, was effective in experimentally-infected rats (3).

when administered orally, even large doses were only moderately suppressive. Parenteral doses were more effective. Inasmuch as the drug persists in the

in 2

It also is noteworthy that the drug has shown chemotherapeutic effect in animals against several parasites other than *E. histolytica*. Time does not permit reviewing the detailed observations along this line except to report that effects can be shown against all of the intestinal amoebae in rhesus monkeys, intestinal trichomonads in dogs, *Trichomonas foetus* and *Trichomonas vaginalis* *in vitro* and in mice and against pinworms in mice.

Toxicity Paromomycin is hardly absorbed at all from the gastro intestinal tract and it is very non toxic when given orally. Mice, rats, monkeys and dogs all tolerated very large amounts of the drug, either when given in one or many doses (2).

oses of
ropor

It remains to be determined whether the parenteral administration of

CLINICAL TRIALS IN INTESTINAL AMOEBIASIS

in extensive trials
Over 400 reports
have been received

Type of patients Among these patients the clinical form of the disease ranged from the chronic cyst passer through the moderately acute to the very acute type of case. Further, the cases reported varied from the moderately well nourished and well nourished in those countries of the higher socio economic level to the anaemic, poorly nourished patients in the countries of a very low socio economic level.

Therapy With the view to determining the minimum effective dosage required the doses of the drug were varied from 5 to 66 mg/kg/day. Generally,

drug but for 3 days

Side effects Three investigators reported that beginning on the second or third day of therapy the number of daily stools was increased by two or three movements. These were soft to loose but never watery in consistency. There was no abdominal cramping, nausea or vomiting and the bowel pattern returned to normal immediately upon the completion of therapy. One patient only of the 432 cases reported had nausea and dizziness; these reactions disappeared when therapy was suspended. The other investigators consistently reported no adverse gastrointestinal effects and no other side reactions from the use of paromomycin.

Summary of investigators' reports (Table I) Paromomycin was used by Carter (6) in the treatment of 104 patients with chronic and subacute intestinal amoebiasis at the Sunland Training Center in Florida. These patients ranged from 2 to 30 years of age and were institutionalized due to epilepsy, mental retardation or spasticity. They were treated with dosages ranging from 5 to 66 mg/kg/day for 15 days. Results were assessed within a few days after the

treated with above 12.5 mg/kg/day all were cleared. The 16 patients treated with 5 mg/kg/day were not cured.

Paromomycin was used in the treatment of 22 patients with subacute and protracted acute intestinal amoebiasis in Panama. Treatment was for a 5 day period using 25 mg/kg of body weight per day. Symptomatic improvement was noted on the second and third day and by the end of the fifth day of treatment all patients were feeling well and their stools were negative for amoebae. Follow up of these cases from 10 to 30 days after the completion of treatment revealed that the patients were still negative for amoebae.

A study in Puerto Rico (7) of 20 cases of chronic and subacute intestinal amoebiasis was made using dosages ranging from 4 to 26 mg/kg/day. The duration of treatment was for 5 days. These patients were followed for a ninety day period at the end of which all remained free of amoebae.

The antibiotic was studied in Florida in 32 children with acute and chronic intestinal amoebiasis. Dosages varied from 10 to 20 mg/kg/day for a 5 day period. An 11 week follow up with stool examinations on the first, second, third, fourth, sixth and eleventh weeks revealed at the end of this period only two recurrences among the 32 patients.

A series of 8 acute cases of intestinal amoebiasis in Honduras were treated with the drug in dosages of 22-28 mg/kg/day for a 3-4 day period. Five of

these patients were completely cured, 2 required a second course of treatment

over two months

Forty one patients with subacute to fulminating acute intestinal amoebiasis were treated in Nicaragua. Treatment was for a 5 day period, 16 patients receiving 12.5 mg/kg/day, 20 patients receiving 25 mg/kg/day and 5 receiving 18 mg/kg/day. In all cases symptomatic relief was obtained on the third or

course of treatment

estimated 95 percent cure rate

In Ethiopia Wagner (9) treated 97 cases of intestinal amoebiasis of the

5 day course of treatment

In South Africa, 10 cases all having ulceration of the bowel, were treated with 2.4 g daily for 10 days. Sixty percent appeared to be cured during a 27-day follow up

and parasitologically negative

Eighteen cases of acute intestinal amoebiasis in the Philippines were treated with 25 mg/kg/day for 3 or 5 days. Of the 17 patients that returned for follow

amoebiasis

THE TREATMENT OF ENTERIC BACTERIAL INFECTIONS

The results of antibacterial inhibition tests *in vitro* and in experimental infections in mice have indicated that paromomycin has significant activity against groups of bacteria associated with enteric infections in man. These bacteria were most notably species of *Salmonella*, *Shigella*, *Paracolobactrum* and enteropathogenic strains of *Escherichia coli* (13). Although definitive clinical trials are still in progress, the present results have shown that orally administered

TABLE I

Paromomycin (Humatin) trials in intestinal amoebiasis (given orally usually 3 daily doses)

Geograph c area	No of patients	Type of infection	Treatment Mg/kg/day	Days	No of pat ents examined and nm negative on days after treatment began					
					1 7	8 14	15 30	31 59	60 & over	
					Exam /neg	Exam /neg	Exam /neg	Exam /neg	Exam /neg	
Chile	15	Chron c	25	5			11/11			
Egypt	20	Acute & Chron c	10-20	7 14	20/19	19/19	19/17	17 15	13/13	
England	19	Chron c	30	10	18/16	18/16	1/1	6/5	4/4	
Ethiopia	97	Acute & Chron c	7 5 30	2 5	79/79	70/70	35/32	96/96	15/15	
Honduras	8	Acute & Chron c	22 28	3 4						
Mexico	26	Acute & Chron c	18 25	5	2/2	26/23	26/23			
Nicaragua	41	Acute & Chron c	12 5 25	5	4/4	31/31	1/1			
Panama	22	Acute & Chron c	25	5						

TABLE I (Contd.)

Paromomycin (Humatin) trials in intestinal amoebiasis (given orally, usually 3 daily doses)

Geographic area	No. of patients	Type of infection	Treatment Mg/kg/day	Days	No. of patients examined and no negative on days after treatment began				60 & over Exam /neg
					17 Exam /neg	8-14 Exam /neg	15-30 Exam /neg	31-59 Exam /neg	
Philippines	111	Acute & Chronic	30	3-5	14/13	5/5	4/4	1/1	
Puerto Rico	20	Acute & Chronic	4-26	5	20/19	20/19	14/14	10/10	10/10
USA	32	Acute & Chronic	10-20	5	29/26	28/28	31/30	25/25	30/29
USA	104	Acute & Chronic	5-66	1-5	36/29	31/23	60/40	19/12	21/14
Union South Africa	10	Acute	2-4 g/day	10			10/6		
Totals	432				222/207	249/234	212/179	114/104	95/85

* Time interval for stool examinations not recorded. However, investigator reported all cases cured.

* Time interval for stool examinations not recorded for five patients. However, investigator reported all cases negative.

** Time interval for stool examinations not recorded. However, investigator reported all cases negative.

paromomycin was effective in controlling human enteritis caused by the aforementioned bacteria. Some representative examples are described in the following section.

Shigellosis and infantile diarrhea An explosive outbreak of enteritis due to *Shigella flexneri* occurred among 160 inmates of a midwestern children's Institution. Approximately half of these patients were given oral paromomycin at a dose of 25 mg/kg/day and half were given 50 mg/kg/day with both groups treated for 6 to 7 days. Only 4 patients from each group failed to respond promptly, and stools from only 2 of these 8 patients yielded *Shigella* on culture (5).

In another study bacteriologic and clinical clearing of shigellosis occurred among 6 to 7 children given oral paromomycin at 50 mg/kg/day for 7 to 9 days. An additional 17 children in the same study were treated with the same regimen of paromomycin for infantile diarrhea caused by various enteropathogenic serotypes of *E. coli*. Fifteen of these 17 patients showed a prompt bacteriologic and clinical response; the 2 patients who did not respond even after 14 days of paromomycin treatment were infected with the 0127 serotype (12).

Salmonellosis Paromomycin was employed for the treatment of *Salmonella typhimurium* infection among a family of 5 in Detroit after 2½ months of treatment with other drugs had failed to eradicate these bacteria from the stools. Four of these 5 patients became bacteriologically negative after 5 days oral dosing with 25 mg/kg/day and the other patient became negative after a second 5-day course at the same dosage. All 5 patients remained well and their stools continued to be free of *Salmonella* throughout a 5-month follow-up period (5).

An additional 33 patients with enteritis due to *S. typhimurium* in a London hospital were treated by McMath *et al.* (11) with oral paromomycin doses of 25 to 75 mg/kg/day for 5 days. Most of these patients had not responded to treatment with other agents. Of these 33 patients 31 patients underwent a bacteriologic conversion subsequent to paromomycin treatment.

Paromomycin was also used by Ross (12) for the treatment of salmonellosis in 9 children 7 of whom no longer had pathogens in their stools after 7 to 9 days oral dosing of 50 mg/kg/day. Ross also reported no side effects and no abnormalities in serial hemograms, urinalyses or blood urea nitrogen and thymol turbidity tests among his paromomycin treated patients.

SUMMARY

Paromomycin is unique among known antibiotics to the extent that it is characterized by direct and marked amoebicidal action and high activity against a wide range of enteric bacteria. It is well tolerated due to almost a total lack of absorption following oral administration. In view of this array of properties as well as the excellent results obtained from more than 1000 patients it would appear that paromomycin will be especially useful for the treatment of amoebic and bacterial enteric infections.

REFERENCES

1. Parke, Davis & Co. Verfahren zur Gewinnung eines neuen therapeutisch wirksamen antiparasitären Stoffes. Austr. Patent 198,888, July 25, 1953.
2. Coffey, G. L., Anderson, L. F., Fisher, M. W., Culbert, I. M. & Hilegaa, A. B. Kolberg, D. L., Thompson, J. E., Weston, K. S. and Ehrlich, J. (1959) *Antib. & Chemother.* 9: 730.
3. Thompson, P. L., Bayles, A. Herbert, S. F., Olczak, B. and McSchnider, J. L. (1959) *Int. J. Antib. & Chemother.* 9: 618.
4. Chang, Y. T. (1959) *Inter. Rev. Tuberc.* 29: 673.
5. Department of Clinical Investigation, Parke, Davis & Co. Unpublished data.
6. Carter, C. H. (1959) *Antib. & Chemother.* 6: 586.
7. Lopez, E. S. F. and Oliver, Gonzalez, J. (1959) *Int. J. Med.* 6: 581.
8. Shale, A. A. (1959) *Antib. Med.* 6: 275.

- 9 Wagner, E. D., personal communication to the Department of Clinical Investigation, Parke Davis & Co
- 10 Dooner, H., *J. Infectious Diseases* (in Press)
- 11 McMath Twining, W. F. and Hussain, K. K. (1959), *Public Health* (London), 73: 328
- 12 Ross, S., personal communication to the Department of Clinical Investigation, Parke Davis & Co
- 13 Fisher, M. W., Manning, M. C., Gagliardi, L. A., Gaetz, M. R. and Erlandson, A. L. (1959-60), *Antib. Annual*, (in Press)

DISCUSSIONS

DR. K. L. ARORA

Q. Will you kindly tell us as to how a physician is to use this drug, if every case is to be judged on individual merit?

Ans. A physician can best answer this question.

DR. O. P. TANDON

Q. What have been the criteria of judging the 'cure' from amoebiasis in this paper?

Ans. A symptomatic phase with absence of *Entamoeba histolytica* in the stool.

DR. R. M. KASLIWAL, Jaipur

Q. This drug is supposed to have nephrotoxic properties as well as it produces diarrhoea. In what way it is an advance on other similar antibiotics (like fumagillin or puromycin)?

Ans. Since it is not absorbed from gastro-intestinal tract there is no oral toxicity. It has not been used parenterally in man. Nephrotoxicity is only a point to be remembered if the drug is to be used parenterally.

NEOVIASEPT IN AMOEBIASIS

R T KAKADE

From the Hyderabad Sanatorium Hyderabad (Dn)

During the past 6½ years I had the opportunity of treating 848 cases of amoebiasis, and the following observations are based on these cases. Various preparations were used for treating these cases and the results were noted comparatively. As far as this paper is concerned the results pertain to neoviassept (Hoechst) tablets only.

Composition

Neoviassept consist of two components (1) Bismuth salt of *p* glycolylamino-phenyl arsonic acid (viassept), and (2) Chloroquine diphosphate. Viassept component is effective intra-intestinally while the chloroquine diphosphate is effective extra-intestinally. The drug is known by name Azalis in America and much work has been done there by Dr D A Barbarian and co-workers and in India by Dr Sarkar Choudhary, Gupte and others.

Selection of cases

All the cases selected for these trials had either *Entamoeba histolytica* vegetative forms or cysts present in the stools. Though some of these cases never presented themselves with cardinal symptoms of amoebiasis they were found to be suffering from this disease.

The total number of cases treated only with neoviassept was 216 of which 106 were males, 61 females and 49 children.

Patients of all ages and both the sexes were found to be suffering equally with this disease. No age seems to be exempt. My youngest patient was 8 months old and the oldest was 76 years of age.

Distribution of cases

1	<i>Alopecia areata</i>	3
2	Cardiac cases	8
3	Ear (Memere's disease)	1
4	Eye	1
5	Fits	6
6	Hepatic involvement	16
7	Intestinal (a) acute	16
	(b) chronic	93
II	Lungs (a) pulmonary	II
	(b) asthmatic	21
	(c) eosinophilic	11
	(d) empyema	2
9	Neuritis and neuralgic	26
10	Skin (a) eczema	4
	(b) vitiligo	4
11	Spongy gums	II
TOTAL		216

(i) *Alopacia areolata*

Three patients presented with the main feature of *Alopacia areolata* patches quite resistant to all other treatment. On examination of their stools *EH* cysts were found and so these cases were treated with neoviasept with full success. Falling of the hair is a common symptom in the amoebiasis patients.

(ii) *Cardiac cases*

These patients come with tachycardia, anginal pains, precordial pain, extra-systole, palpitation, low blood pressure, fainting attacks etc.

(iii) *Meniere's disease*

A case diagnosed as Meniere's disease was referred to me. On a casual examination of the stools *EH* cysts were found. The patient responded very well to anti-amoebic treatment with neoviasept and vitamin B-complex therapy.

(iv) *Eye*

A case of Iridocyclitis was referred for a check up by the ophthalmologist as the patient had *EH* cysts in his stools. The ocular lesions improved with neoviasept treatment.

(v) *Fits*

Some cases of idiopathic fits were examined and found positive for *EH* cysts. These cases were relieved of their fits with anti-amoebic treatment.

(vi) *Hepatic involvement*

It is the commonest complication of amoebiasis. Some patients had severe pain in abdomen and in right shoulder joint up with high fever and leucocytosis. Some had developed the hepatic abscess and some abscesses burst into thoracic cavity giving rise to empyema. I could get the best results in these hepatic cases with neoviasept tablets.

(vii a) *Acute intestinal amoebiasis*

The diagnosis in these cases is quite easy. Vegetative forms are generally found in these cases. These cases responded very well to neoviasept in conjunction with emetine hydrochloride and vitamin B-complex.

(vii b) *Chronic intestinal amoebiasis*

The symptoms vary from simple heaviness and dull ache in the abdomen to acute abdominal catastrophe simulating acute appendicitis, perforating gastric ulcer, biliary colic, renal colic, etc. Loss of appetite, lassitude, forgetfulness, loss of concentration, feeling of uneasiness, growing abdomen, constipation, dyspepsia etc., are the commonest symptoms. Onset in many cases is insidious and patient is unaware of the infection he is harbouring. Only 38% of the cases come with abdominal symptoms.

(viii) *Lungs*

(a) *Asthamatic attacks*—Some patients come with paroxysmal attacks of cough as their main symptom. Breathlessness, wheezing, difficult breathing, cyanosis make the picture simulate 'Status Asthmaticus'. Perhaps this is an allergic reaction to a foreign protein produced by the presence of *EH* cysts. These cases cleared up after treatment with neoviasept, i.e., *EH* cysts was eradicated. Erythrocyte count ranging from 10000 to 15000, eosinophilic count ranging from 10% to 20%, and eosinophilia were found.

to be positive for *EH*. Sometimes it takes months to find *EH* cysts in the stools of such patients. One very interesting finding I came across was that,

stool was also negative in both these cases and later *EH* cysts were found in stools. Patients were treated on the anti amoebic line and the X rays taken six months later were quite clear. In some cases where the infection has reached the liver give rise to the right basal infiltration due perhaps to the upward rise of the liver and congestion of right base which in many a times wrongly diagnosed as the cases of right basal lesion of tuberculosis.

Two cases of amoebic empyema on the right side were treated by aspiration and anti amoebic line of treatment with neoviassept and emetine with very good results.

(ix) *Neurasthenia, neuralgia and neuritis*

This group of cases was present in a large number. Patients generally come with these as the main symptoms and do not have any other trouble. Any nerve in the body may be affected such as trigeminal, facial, sciatic or some small peripheral nerve. I have now made it a rule to examine the stool of every such patient as a routine. These symptoms are probably due to the toxin produced by the cysts in the body. Tobacco in any form aggravates these nervous symptoms and better results are achieved by asking the patients to stop smoking or chewing tobacco. While treating these cases satisfactory results are achieved if the therapy is combined with massive doses of vitamin B.

(x) *Skin*

The commonest and allergic phenomenon seen is eczema. Eczema with amoebiasis responds very well to neoviassept course with vitamin C. Another very interesting finding is vitiligo with raised border.

(xi) *Spongy gums*

Chronic amoebic cases have yet another common symptom i.e., spongy and bleeding gums. Very resistant cases which did not respond to massive doses of vitamin C, calcium, vitamin A and K but responded very nicely to anti amoebic treatment with neoviassept.

Dosage

- 1 *Male* For male adults two tablets three times a day for 12 days was quite effective though some of my colleagues advocated six tablets a day for 8 days.
- 2 *Female* Four tablets a day for 12 days.
- 3 *Child* Three tablets a day for 12 days.

As already mentioned above better results are achieved if vitamin B is also given in conjunction with neoviassept.

After the 12 days course a gap of 2 weeks is given and stools are examined for *EH* cysts. If the stools are found positive the same course of 12 days is repeated. The stools are repeatedly examined for the next six months to two years at the interval of a fortnight and if still the patient be found positive, he was given the third course of neoviassept for another 12 days. The very resistant cases required the third course.

The following table will show the results of the cases and the number of courses given to them

TABLE I

Table showing number of courses given for complete cure

Sex	1st course	2nd course	3rd course
Males	106	III	4
Females	61	9	3
Children	49	11	0
Total	216	36	13

Follow-up

Out of 216 cases, 138 cases could be followed for the following time

Cases followed for 18 months	74
Cases followed for 2 years	36
Cases followed for 3 years	16
Cases followed for 4 years	8
Cases followed for 5 years	4

How-
l com-
te was

the minimum and the percentage of cure is the highest Not a single case was found to be positive after giving the third course of neoviassept

Side-effects and after effects

Side-effects and after-effects were practically negligible. Some of the patients complained of nausea, loss of appetite, mild degree of pain in abdomen, headache and very rarely vomiting, if neoviassept tablets were given alone. When given in conjunction with vitamin B complex, no patient ever complained of any side-effects.

The follow-up shows no toxic effect of the drug even after giving the three courses to the patients.

CONCLUSION

The trials conducted with neoviassept, other anti amoebic drugs and anti-biotic drugs for amoebiasis show that the best results are achieved with neoviassept in acute as well as in chronic amoebiasis, may it be intestinal or extra-intestinal. Acute cases respond very well if the therapy is combined with emetine and vitamin B-complex. Chronic cases (intestinal and extra-intestinal) respond very well with neoviassept combined with vitamin B. About 16% required course to be repeated twice and 5% required course to be repeated three. No person required more than three courses when treated with neoviassept.

INIOBEN IN AMOEBIASIS

P. K. GHOSH AND S. GUPTA

From the Mayo Hospital Calcutta

examined in a general hospital contained *E. histolytica* infection—or one in every 5 stools

In a similar bigger hospital in a semi urban area (Burnpur) among 4211

hospitals much more male patients attend than female patients

The age distribution among our 85 cases are as follows

0—10 yr	20
11—20	16
21—30	28
31—40	18
41—50	1
51 & above	2

Thus the infection is almost equally prevalent in all ages up to 40 yr in urban area. Infants and children are no exception they constitute 23.5% or nearly quarter of the cases in the series. There are 2 cases aged 1 yr and 11 cases upto the age of 5 yr. Seventyfive percent of the cases were in persons up to the age of 30 yr.

The presence of other bowel infections It is difficult to prove that *E. histolytica* favours other infections by lowering local bowel resistance—but other protozoal and helminthic infections are associated. The latter are rather concomitant than result of *E. histolytica* infection. In urban areas the association with giardiasis is remarkable—94 cases (29%) in 322 amoebic stools. In semi-urban and rural areas giardiasis is less common—10 cases of helminthic infection only among 12 amoebic stools (nearly 8%). Whether *E. histolytica* has harmful effect on normal bacterial flora engaged in synthesis of vitamin B complex in the intestines in the same way, as has been noticed after the use of some antibiotics, remains yet to be worked out. Possibly it does, for the dyspeptic symptoms and bowel disorders are akin to symptoms of B-complex deficiency.

ENTOLOGY

E. histolytica causes acute and chronic bowel disorders. Emetine is beneficial in acute cases. Chronic cases are difficult to cure and continue to recur. Many preparations are used in their treatment, e.g., *Kurchi*, *violform*

In *in vivo* (rat) marked amoebicidal action is noticed with 50-75 mg/kg by mouth for 5 days. In larger doses it produces vomiting and minute hematuria in animals (dogs). In 600 mg daily dose in man it induces vomiting. Therapeutically

plus
primary stool examination—no case was included where L.H. cysts or vegetative forms were not found. The cases were all ambulatory except five. The dose given in adults was one tablet 3 times a day for 10 days, or 2 tablets 3 times a day for 3 or 4 days, then 1 tablet 3 times a day for 6 or 7 days. Children received 1/2 tablet 3 times a day. The stools became cyst-free in 7-10 days on an average, and in some cases () with 6 tablets a day. Vegetative forms and trachemata. Stools were examined after the institution of treatment—and

upto 4 weeks in some instances.

There were 52 male and 11 female cases. The lowest age was 1 yr and highest 71 yr. Cysts were present in 35 (55.5%), vegetative form in 20 (31.7%) and both forms in 8 cases (12.8%). In 6 samples giardia cysts, and in another 8 samples ova of hookworm and/or roundworm were also found.

The total number of days of treatment was 583 days for 63 cases, or 9.2 days per case. Twenty-eight cases (44%) were treated for 10 days, 27 (43%) for 11-18 days, and 8 (13%) for 4-6 days.

RESULTS

Clinical relief (cure) was obtained in all 63 cases. The cysts persisted in stools in 3 cases—in all others the cysts had disappeared.

Giardia cysts
not seen here

numbers, of giardia from the stool. But it had no effect on ova of hookworms. Enlarged and/or tender liver also improved and became smaller.

There were 3 cases of mild nausea and one case of allergic dermatitis (scalp) in this series. Eleven cases suffered from constipation. There were no other toxic manifestations.

In the second series entobex alone in 50 mg doses was tried in 25 cases. Three cases did not complete the course of treatment. In the final analysis there were

1 and 55 yr

yr range

continued f

usually 7, 10 and 15 days after commencement of treatment. Results of treatment was considered good in 13 cases (59%) there was clinical cure and the cysts

(32%) In 7 cases

the re 2 cases (9%

appea —cysts dis

Seven cases (28%) complained of constipation; there were no other toxic mani-

festations

CONCLUSION

above toxic manifestations were absent. It is thus both a safe and potent drug. Besides its anti amoebicidal action it seems to be active also on giardial infection.

The presence of other bowel infections It is difficult to prove that *E. histolytica* favours other infections by lowering local bowel resistance—but other protozoal and helminthic infections are associated. The latter are rather concomitant than result of *E. histolytica* infection. In urban areas the association with giardia is remarkable—9½ cases (29%) in 322 amoebic stools. In semi-urban and rural areas, giardiasis is an intestinal infection only among 127 cases. Giardiasis has harmful effect on normal B complex in the intestines in the same way, as has been noticed after the use of some antibiotics, remains yet to be worked out. Possibly it does, for the dyspeptic symptoms and bowel disorders are akin to symptoms of B complex deficiency.

ENTOBEX

E. histolytica causes acute and chronic bowel disorders. Emetine is beneficial in acute cases. Chronic cases are difficult to cure and continue to recur. Many preparations are used in their treatment, e.g., *Aurchi*, *ioform*,

mouth for 5 days. In larger doses it produces vomiting and minute hematuria in animals (dogs). In 600 mg daily dose in man it induces vomiting. Therapeutically 300-400 mg is a safe dose.

In one series of 63 cases of intestinal amoebiasis, the effect of entobex (20 mg) plus enterovioform (200 mg) was studied. Every case was selected after a preliminary stool examination—no case was included where EH, cysts or vegetative forms were not found. The cases were all ambulatory except five. The dose given in adults was one tablet 3 times a day for 10 days, or 2 tablets 3 times a day for 3 or 4 days, then 1 tablet 3 times a day for 6 or 7 days. Children received correspondingly lower doses. The stools became cyst free in 7-10 days on an average with 3 tablets a day—earlier (4-5 days) with 6 tablets a day. Vegetative forms also disappeared in 3-5 days with the latter schemata. Stools were examined 4 or 5 times in each case upto 3 weeks from the institution of treatment—and upto 4 weeks in some instances.

There were 52 male and 11 female cases. The lowest age was 1 yr and highest 71 yr. Cysts were present in 35 (55.5%), vegetative form in 20 (31.7%) and both forms in 8 cases (12.8%). In 6 samples giardia cysts, and in another 11 samples ova of hookworm and/or roundworm were also found.

The total number of days of treatment was 583 days for 63 cases, or 9.2 days per case. Twenty-eight cases (44%) were treated for 10 days, 27 (43%) for 11-18 days, and 11 (13%) for 4-6 days.

RESULTS

Clinical relief (cure) was obtained in all 63 cases. The cysts persisted

Formed *C. parvum* cysts

There were 3 cases of mild nausea, and one case of allergic dermatitis (scalp) in this series. Eleven cases suffered from constipation. There were no other toxic manifestations.

In the second series entobex alone in 50 mg doses was tried in 25 cases. Three cases did not complete the course of treatment. In the final analysis there were thus only 22 cases (13 male and 9 female). The ages varied between 1 and 55 yr (7 in 0-10 yr, 9 in 11-20 yr, 3 in 21-30 yr, 2 in 31-40 yr, and 1 in 55 yr range). The dose was either 1 tablet 3 times daily or 2 tablets 3 times daily, and continued for 10 days. Besides the preliminary examination stool was examined usually 7, 10 and 15 days after commencement of treatment. Results of treatment was considered good in 13 cases (59%); there was clinical cure and the cysts were negative on two examinations on the 10th and 15th day. In 7 cases (32%) there was clinical improvement, but the cysts persisted. In 2 cases (9%) the result was considered doubtful—there was clinical improvement—cysts disappeared on second examination, but reappeared on the third examination. Seven cases (33%) complained of constipation. There were no other toxic manifestations.

CONCLUSION

Entobex is a potent anti amoebicidal drug. In 59% of cases it cured the disease and caused disappearance of cysts from the stool. In combination with enterovioform its action seems to be further enhanced. Thus in 63 cases where this combination was tried, 60 cases responded favourably. In the doses given above, toxic manifestations were absent. It is thus both a safe and potent drug. Besides its anti amoebicidal action it seems to be active also on giardial infection.

TREATMENT OF AMOEBIASIS

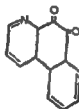
A CLINICAL TRIAL OF NEW SYNTHETIC COMPOUND—11925-C*

J G PAREKH AND BABUBHAI D PATEL

From the J J Group of Hospital, Bombay

Amoebiasis is common in our country. The last word in its treatment has yet to be said. Fairly large number of drugs are available, each one with a variable effect on one or the other stage of the parasite. Most of these drugs cause disappearance of the symptoms and of the parasites, at least temporarily. Relapses and reinfections, however, are common but it is difficult to differentiate them or prevent them and the search for better drugs continues.

A new synthetic compound 11925-C, 4-7-phenanthroline-5-6-quinone,



I

Microbial interrelationships are involved in the aetiology, pathogenesis and pathology of intestinal amoebiasis. By itself in absence of bacterial flora, *E. histolytica* appears not only incapable of producing the disease but it does not even survive for more than a few days. A synergism of amoeba and bacteria is a pre-requisite to the development of the disease (2).

Since 11925-C is claimed to possess a dual action both anti-amoebic and antibacterial it could be expected to prove to be a better drug than the existing amoebicidal drugs.

It is slightly soluble in water and it is rapidly absorbed. It is excreted in bile and urine (1). It is relatively free from toxic effects in animals. In men, except for occasional nausea and vomiting, and some minor toxic effects, e.g., headache, diarrhoea, petechial rash, etc., no serious toxic effects have been reported (3-6).

The compound, 11925-C, has been under clinical trial all over the world for over 11 years and several reports have appeared in this country (3, 7-10).

This paper deals with a clinical trial of this new phenanthroline derivative in amoebiasis.

* Entobex (Ciba)

MATERIAL AND METHODS

A series of 135 adult patients attending J J Hospital (out patients department) were taken up for the present study, on finding the vegetative and/or cystic forms of *E. histolytica* in the stool examination.

A detailed history of the complaints and past history of diarrhoea, dysentery or gastro enterological disorders were recorded. The patient's physical examination was carried out in details. The stools were examined usually on alternate days during the first 10 days of the treatment and then at the end of a fortnight, a month, and 2 months in as many cases as possible. Sigmoidoscopic examination was carried out before and after the treatment in a very small number of cases. At the same time clinical progress was noted.

After therapy, the stools were examined with few exceptions by merthiolate iodine formaldehyde concentration method (Dave), to ensure greater accuracy in assessing the results.

For the purpose of treatment, the patients were divided in three groups. The first group received the respective treatment for a period of ten days. No other anti amoebic treatment was given.

FINDINGS

The common symptoms are pain in abdomen (71%) and

TABLE I

Clinical manifestations

	Total	Percentage
Total cases	135	
Acute dysentery	17	13
Chronic dysentery	90	66
Asymptomatic cases	12	9
Pain in abdomen	96	71
Abnormal bowel movements	127	93
Frequency of stools	33	24
Irregular bowel habits	52	38
Constipation	41	31
Previous history of diarrhoea or dysentery	79	58
Enlarged tender liver	2	

Mexaform (CIBA)

The results of microscopic examination of the faeces in the 135 cases are presented in Table II.

TABLE II
Findings on examination of faeces

	Total	Percentage
Total number of cases	135	—
Cyst of <i>E. histolytica</i>	118	82
Vegetative <i>E. histolytica</i>	7	5
Vegetative & cystic <i>E. histolytica</i>	10	7
More than one parasite	76	56
Cyst of <i>E. coli</i>	33	24
Cyst of <i>G. lamblia</i>	21	14
Cyst of <i>I. butchli</i>	3	2
Ova of ascaris	21	15
Ova of trichuris	22	14
Ova of ankylostome	5	3.5

RESULTS OF TREATMENT

The criteria adopted for the successful therapy were the clinical improve-

cases

TABLE III
Results of therapy

	Group I	Group II	Group III	Total
Number of cases	38	37	40	135
Cases completely studied	28	36	32	96
Immediate cure	25	29	31	86
Percentage immediate parasite clearance	89	80	100	88
Relapses	3	4	5	12.5%
Percentage of final parasite clearance	78	69	84	77

Of the 135 cases 96 could be adequately followed. Eighty-six (88%) had immediate parasite clearance. Seventy-four (77%) had both—symptomatic relief as well as parasite clearance at the end of the follow-up. Acute manifestations responded better than chronic manifestations. *E. coli* and *G. lamblia* were also favourably affected.

Groupwise, the group III in which the combination of 11925 G (50 mg) with enterovioform (250 mg) 3 times a day was used, gave best results having 100% parasite clearance at the end of ten days' therapy. While 27 cases, i.e., 81% were cured as observed during the follow-up.

In group I, treated with 11925-G alone, out of 28 cases, 25 (89%) were free of

E. histolytica at the end of 10 days while 22 cases (78%) were cured in the later follow up

In group II, of the 36 cases, 29 (80%) did not pass *E. histolytica* at the end of therapy, while 23 (69%) were completely cured as seen by the later follow up

DISCUSSION

bowel movements, chronic dyspepsia were other common features. Two patients had enlarged tender liver

Multiple parasitic infestation was a common feature. Some cases had as many as 4 to 6 varieties of parasites (both protozoa and helminths). As many as 56% had more than one parasite. Most common accompaniment was *E. coli* (24%)

In view of the lack of complete satisfaction regarding the treatment of amoebiasis, attempts have been made to produce newer synthetic drugs to bring about better results. Compound 11925 G is one such drug. Out of 135 cases taken up for this study, 96 could be adequately followed for a period of two months. The results of our study indicate that 11925 G produces immediate

It also influences *G. lamblia* as given slightly better results 1 combined with 750 mg of enterovioform, the results were still better but not statistically significant. There were no serious side effects in any of the patient, except two cases who had abdominal pain during the therapy

Sagone (5) treated 720 patients of amoebiasis with 11925 G for 11 days and repeated the course after 2-3 months. He obtained stool clearance in 92.5%

He used on an average 30 mg daily. In Nagaty, *et al* (11) estinal amoebiasis in 12 mg per kg body

then lower doses were

vachdev (6) r kg body

7) observed

In Sagon's e still stool

al clearance ver, 30.1%

were stool positive after three months. The author, nevertheless, considers 11925 G to be more effective than emetine, well tolerated and a better amoebicide than many now in use. He also found it encouraging in the treatment of other intestinal protozoal infections

Its action on amoebic hepatitis is controversial as reported by some authors (4, 8-10, 12)

the fact that 11925 G was used as a gauge of the value of the drug, for eradication of therapy could not be used as a gauge of the value of the drug, for eradication of

the infection. Any contact amoebicide may render the stool negative temporarily as mentioned by Adams (3).

SUMMARY AND CONCLUSIONS

This study presents the results of a clinical trial given to a new synthetic phenanthroline quinone derivative in 135 cases of amoebiasis. 96 cases could be studied adequately.

The cases were divided in three groups. The treatment was given for ten days. In group I, the patients were given 50 mg. of compound 11925-G three times a day.

Out of 28 cases, 25 (89%) were amoeba free at the end of therapy and 22 cases (78%) were free at the end of the follow up.

In group II, the patients were put on 20 mg. compound 11925-G + 200 mg. enterovioform three times a day.

Of the 36 cases, 29 (80%) were amoeba-free at the end of therapy while 25 (69%) were free at the end of follow up.

In group III, patients were given 50 mg. 11925-G and 250 mg. enterovioform three times a day.

Out of 32 cases studied, all (100%) cases were amoeba-free at the end of therapy but 5 cases again showed the parasites during the follow-up, thus 27 (84%) were free.

Thus the best results were obtained in group III with compound 11925-G giving in the dose of 50 mg. along with enterovioform 250 mg., both three times a day, for ten days.

Two cases of amoebic hepatitis patients in group II did not respond satisfactorily.

The incidence of *E. coli* and *G. lamblia* was also reduced after the course of treatment.

The drug was found to be well tolerated, non-toxic and with high therapeutic index.

The clinical and amoebicidal effects compare favourably with the currently used drugs.

ACKNOWLEDGMENTS

Our thanks are due to the Professor of Pathology, Dr. R. K. Gadgil, the

to carry out this work and publish this paper.

We are thankful to Dr. B. L. Silveira, Manager, Clinical Research Division, M/s. CIBA Pharma Private Ltd., Bombay for supply of the drugs used in this trial.

REFERENCES

J. Med. Hyg. 7: 392

Ht, W. H. and Regniers J. A.

7: 743

7. A. L. L. A. J. H. C. M. / D. D. Z. F. A. M. J. A. 41, 31

329

12. Chatterjee, P. K., Mukherjee, S., Ghosh, S., Das, A. K., Das, S. K.,
Med Assoc, 30, 251

Jan

CLINICAL TRIAL WITH PHENANTHROLINE COMPOUNDS IN THE TREATMENT OF CHRONIC AMOEBIASIS

P. K. CHATTERJEE

Calcutta

Two phenanthroline compounds namely, 4,7-phenanthroline, 5,6-quinone (Entobex or 11925C) and its semicarbazone (11925) have now been given a fair trial by a number of Indian workers, Sen *et al* (1), Sen and Sanyal (2), Roy Chowdhury *et al* (3), Chatterjee *et al* (4), Das (5), and Sachdev and Duttar (6). Both the compounds were found to have strong amoebicidal action *in vitro* as well as bactericidal action, entobex being twice as strong as its semicarbazone derivative (personal communication from the scientific department of CIBA Pharma). The amoebicidal action of the compounds *in vivo* was also studied in experimental amoebiasis of rats and was found satisfactory. In animal experiments the compounds were found to be well tolerated.

MATERIAL

Since 1957 a total of 127 patients have been treated with the compounds. Of these 9 were treated for infections other than amoebiasis, 22 patients did not complete the course and in 4 patients treatment had to be abandoned due to severe intolerance. Excluding these 35 cases, a total of 92 patients considered to be suffering from chronic amoebiasis were treated with a course of these compounds.

Patients with chronic or recurrent diarrhoea with mucus in stool, vague discomfort or pain in abdomen, palpable and tender caecum, showing *E. histolytica* in their stool, in cystic or vegetative forms, either alone or with giardia, were selected for treatment. A few cases were also included in the trial, although a single examination of their stool was negative, because they showed the above symptom complex and no other obvious cause for the symptoms could be found. These were considered as clinically diagnosed cases and their results were considered separately. Patients with other vague abdominal symptoms such as anorexia, flatulence, irregular bowels, etc., were also selected for treatment if their stool examination showed presence of *E. histolytica* and no other obvious cause for their symptoms could be found.

Sixty-nine cases had positive stool (group I) and 23 cases were clinically diagnosed (group II). Most of the patients were between 20 and 40 years of age. About half the patients were attending the out-patient department during treatment and the rest were in hospital at bed rest.

TREATMENT

The compounds were given in tablet form by mouth. One group was treated with 11925 (semicarbazone derivative) (100 to 200 mg) 3 times a day, another group was treated with entobex (11925C) (100 mg) 3 times a day, a third

group was treated with a combination of entobex and enterovioform. In this last group four different dosage schemes were followed

	<i>Entobex</i>	<i>Enterovioform</i>
Scheme I	50 mg twice daily	250 mg thrice daily
Scheme II	50 mg thrice daily	" " "
Scheme III	40 mg " "	400 mg " "
Scheme IV	20 mg " "	200 mg " "

RESULTS

In assessing improvement in character taken into account stools were positive or not

Group I (with positive stool)	69 cases
Group II (clinical cases)	23 "

In group I 25 cases were treated with 11925 (semicarbazone derivative) and the clinical improvement was seen in all of them. One case had acute dysenteric symptoms which were controlled in 5 days. Chronic loose stools with mucus were present in 13 cases. In all the cases stools improved in character and number in 3 to 6 days. At the end of 10 days treatment *E. histolytica* disappeared from stools in 23 of the 25 patients. In 3 patients liver was enlarged and tender before treatment. In all the 3 cases there was marked improvement after 10 days treatment. In 2 cases although some clinical improvement occurred stools were still positive after 10 days' treatment. Most of the cases were treated

dose had to be reduced

In the 11 cases where a day was given

Eleven cases of group I were treated with entobex. One of them had acute dysenteric symptoms and four had chronic loose stools. In all these 5 cases diarrhoea was controlled and stools improved in character in 3 to 6 days. In

no appreciable

the remaining

or dysentery

given in doses

of 100 mg 3 times a day. In those cases who failed to respond an increase of

success

one third of the

were severe with

or treatment dis

continued. In 4 cases originally selected for treatment with 11925 treatment had to be abandoned due to serious symptoms of intolerance. With entobex symptoms of intolerance were much milder and treatment could be continued in all cases.

In view of these facts it was decided to try entobex the better tolerated

Twenty-five cases of group I showed marked improvement in clinical signs and symptoms as well as in character of stool. In 19 of these cases stools were examined after completion of treatment and were found negative in 15. In 4 cases stools were still positive after treatment. In Group II, 17 of the 23 cases showed marked improvement.

As mentioned before four different dosage schemes were followed in the combined treatment. The results under these schemes are shown separately in Table I.

Group I—Cases with positive stool

Group II—Cases diagnosed clinically

On the individual symptoms in the two groups acute dysenteric symptoms were relieved promptly in 3 out of 13 cases and chronic loose stools were controlled in 17 of 25 cases. Enlarged and tender liver was present in 13 cases. In one case only marked improvement was seen and in 6 cases only slight improvement occurred.

TABLE I

Dosage scheme	Group I			Total treated	Group II	
	Total treated	Improved	Not improved		Improved	Not improved
I	5	5	0	4	2	2
II	3	3	0	1	1	0
III	13	11	2	9	8	1
IV	12	6	6	9	6	3
Total	33	25	8	23	17	6

In the combined treatment the dose of entobex varied from 150 mg. to 600 mg. per day. From Table I it will be seen that all the cases treated under schemes I and II showed marked improvement in group I. But in scheme IV with 600 mg. of entobex and 600 mg. of enterovioform per day the results were less satisfactory than with scheme III with double the dose of the two drugs.

Tolerance. Both the compounds were well tolerated. Only in higher doses symptoms of intolerance, mainly gastro intestinal irritation, were seen. There seemed to be considerable individual variation in tolerance. While some patients could tolerate even 600 mg. of 11925 and 11925C a day, others had trouble with even 150 mg. a day only. The symptoms of intolerance seen were anorexia, nausea, vomiting, dryness of mouth, diarrhoea and burning in the eyes. Roughly, one third of the patients showed some symptoms of intolerance. The entobex was better tolerated and symptoms of intolerance were mild. Of all the cases originally selected for treatment intolerance was severe enough to abandon treatment in 4 cases only. All these cases were treated with the compound 11925.

DISCUSSION

Results of treatment of chronic amoebiasis are notoriously difficult to assess. While immediate relief of symptoms and disappearance of parasites from stools may occur with many drugs relapse rate is high. In a short term trial therefore it is not possible to assess the value of a drug.

involving absence from work. Most physicians in general practice, therefore,

From the Hoffmann La Roche & Co Ltd, Basle, Switzerland

CHEMISTRY

Osborn
ties, b-
easily

TABLE I

LD mouse	Emetine			Ro 1 9334		
	oral	s c	i v	oral	s c	i v
10%	28	28	14	35	58	24
50%	35	35	18	50	70	28
90%	43	43	22	70	88	35

Emetine thus seems to be almost twice as toxic as Ro 1 9334

Subacute toxicity

normal but both preparations produced a slight neutropenia with relative lymphocytosis

The histologic studies of the organs of animals receiving Ro 1 9334 revealed only a slight turbid tumefaction of the liver and a diminution of lymphatic tissue in the spleen

Chronic toxicity

contained in Table II

TABLE II

Amount contained in feed (%)	Ro 1 9334		Emetine	
	Amount absorbed daily (mg/kg)	Effects	Amount absorbed daily (mg/kg)	Effects
0.05	11.2	All animals dead after 1 week	30*	All animals dead after 1 week
0.01	4.5	All animals dead after 2½ weeks	6*	All animals dead after 2 weeks
0.005	3.5	All animals survived 12 weeks	3	All animals dead after 2 weeks

*Dose estimated ilico rectally

and emetine even at the lowest daily
weeks are still highly toxic. How-
ever, while those receiving emetine
is better tolerated. Furthermore,

the animals receiving Ro 1-9334 showed a weight loss of $\frac{1}{2}$ to $\frac{1}{4}$, slight turbid tumefaction of the liver and myocardium, but no alteration in other organs or the blood count

PHARMACOLOGY

Intravenous administration of the compound Ro 1-9334 in doses of 2, 5 to 5 mg/kg produces transient hypotension in the cat anesthetized with Numal. In doses of 5 and 10 mg/kg it provokes a prolonged decrease in the blood flow of the carotid and femoral arteries. These effects are less intensive than with emetine.

In doses of 10 mg/kg Ro 1-9334, like emetine, reduces the pulse rate by 12% during 10 to 15 min.

The effect of Ro 1-9334 and emetine on the electrocardiogram was studied on rats by intravenous infusion at constant volume and speed of differently concentrated solutions. In these acute toxicological trials, where 1.8 to 0.18 mg/kg per minute were injected, appeared in both substances marked electrographic changes consisting in abnormal ventricular complexes, a-v lengthening, extrasystoles, extreme bradycardia. However, in order to provoke death by cardiac arrest in 5-10 min about the double doses of Ro 1-9334 compared with emetine were necessary. Both the substances in 0.18 mg/kg per min could be injected during 120 min without altering the basic pattern of the electrocardiogram.

The respiratory volume increases by 20% after i.v. administration of 5 mg/kg and by 80% after doses of 10 mg/kg. These effects last 3 and 2 min respectively, and are followed by a reactional decrease during 11 min. Emetine has a rather depressive action in similar conditions. In concentrations of 2×10^{-4} and 2×10^{-5} , Ro 1-9334 relaxes the spasmodic effect of BaCl_2 and in a concentration of 2×10^{-4} also that of acetylcholine. The same concentration of the compound stimulates the isolated uterus of the rabbit and reduces the action of adrenaline. The effects do not seem to be specific.

The emetic effect of Ro 1-9334 and emetine was studied on the dog after oral and subcutaneous administration. After 1 mg/kg oral dose, emetine produced 56 retching in 45 to 170 min, while Ro 1-9334 produced the same oral dose of 56 retching in 45 to 170 min as well as subcutaneous.

EXPERIMENTAL CHEMOTHERAPY

Antiamoebiasis activity

the concentration and the oral dose. It can

TABLE III

Drug	LD ₅₀ mouse mg/kg oral	LD ₅₀ mouse mg/kg s.c.	In vitro γ/cc end point	In vivo CD rats mg/kg
Ro 1-9334	50	70	1:100	1.25—3.85
(—) Emetine, synthetic comp. equivalent to natural emetine	35	35	1:100	3.1—7.8
(+) Emetine, synth. comp. corresp. to the opt. isomer of nat. emetine	—	700	1:1000	170

be seen that Ro 1-9334 and (—) emetine the natural alkaloid, have similar toxicity and *in vitro* activity, but that the *in vivo* activity of Ro 1 9334 is about twice that of emetine (+) Emetine is atoxic but inactive

Action against Schistosomiasis mansoni of the mouse

Daily treatment of mice with 10 mg/kg of Ro 1-9334 for a fortnight beginning 32 days after infection with Cercaria of *Schistosoma mansoni* provokes a significant reduction in the number of worms in the liver and in the mesenteric and portal veins. The effect is almost the same whether administration is oral, subcutaneous or intraperitoneal

PRELIMINARY CLINICAL RESULTS

The clinical trial of Ro 1 9334 began a year ago in a limited number of medical centres. The experience gained does not yet allow definitive conclusions. However, some provisional statements can already be made

Amoebiasis

About 40 cases of acute hepatitis and hepatic abscess, and 20 cases of acute intestinal amoebiasis have been treated so far with subcutaneous or intramuscular injections of the 10% solution of Ro 1 9334. The drug has shown a distinct therapeutic effect and the cure rate obtained seems to be of the same order as the one of emetine

As these cases were the first patients treated and it was not known how well the drug would be tolerated, the dosage employed was rather low and not uniform (30-80 mg daily over 10 days). This could explain the few cases which did not respond as promptly as they might have done to emetine

Schistosomiasis

Ro 1 9334 has shown an evident effect on about 60 patients with *Schistosomiasis mansoni* (6-7). The parasitological cure rate appears to be 40-60% with the rather low dosage so far employed. Results will probably improve with the higher doses now used (80-120 mg daily over 10 days). Trials have also started on *S. haematobium* and *S. japonicum*

Tolerance

Systemic side effects were infrequent after doses of 0.5-1.0 mg/kg over 10 days and somewhat more frequent but never alarming, after 1-2 mg/kg. Thus Ro 1 9334 seems to be better tolerated by the cardiovascular and neuromuscular system than emetine

associated with clinical symptoms

Headache was observed in 3 cases, nausea in 4, vomiting in 3, abdominal pain in 8, diarrhoea in 7 and muscular pain in 4 cases

Local tolerance of Ro 1 9334 seems to be better than that of emetine. In some cases the patients complained of the volume of liquid injected. A 20% solution is therefore being prepared

To investigate the possibility of giving Ro 1 9334 *per os*, acid resistant-coated tablets were administered to a limited number of patients. In spite of evident activity against *Schistosomiasis mansoni*, amoebiasis and isolated cases of infestation

with *Trichuris*, *Ascaris* and *Hymenolepis* the trials had to be stopped because of frequent nausea vomiting and diarrhoea. A new form of tablet is under study.

SUMMARY

Chemical pharmacologic toxicologic and chemotherapeutic properties of Ro 1 9334 (2 dehydro emetine), a new synthetic compound are briefly discussed.

As preliminary clinical trials in amoebiasis and schistosomiasis showed promising results, further clinical investigation of this drug is suggested.

REFERENCES

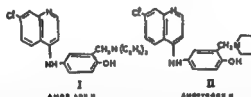
- 1 Osbond I M (1959) *Chem Ind* p 257
- 2 " " " " " " " " " " " " " " " "
- 3 " " " " " " " " " " " " " " " "
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ANTI-AMOEBIIC AGENTS PART VI PROMISING BASIC AMOEBIICIDES DERIVED FROM 5 CHLORO 8-QUINOLINOL

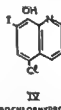
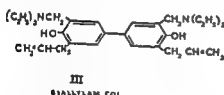
J H BURCKHALTER, WILLIAM S BRINIGAR¹ AND PAUL E THOMPSON

From the School of Pharmacy, University of Kansas, Lawrence, and the Research Division of Parke, Davis & Co., Detroit, Michigan

During the past fifteen years, we have been interested in the synthesis of phenolic Mannich bases as antiprotozoan agents. Amodiaquin, (camoquin)



administration (8). Ballylamicol (camoform) (III) was originally synthesized as an antimalarial (9) and it was found to be an effective agent against blood-induced human malaria (10). However, because of its activity against both intestinal and extraintestinal amoebiasis (11) it is being produced as an anti-amoebic agent.



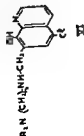
Iodochlorohydroxyquin (violet form) (IV) is illustrative of the 8-hydroxy-quinoline amoebicides whose activity is limited to the intestine (12). In an attempt to convert the insoluble type IV to an agent which might possess systemic

¹ Previous publication: Nobles, W. L., Stephens, V. G., Wei, L. and Burckhalter, J. H. (1958), *J. Amer. Pharm. Assoc.*, 47:82.

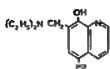
² Parke, Davis & Co. Fellow. Chemical part was taken from his Ph.D. Thesis, (1957) University of Kansas, U.S.A.

TABLE I

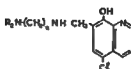
5-Chloro 7 (diethylaminoethylammoniumethyl) 8-quinolinsulfate



VI	R ₁ N	n	Proce dure	Y eld (%)	M p (°C)	Formula	Analyses			H	Fo ind	Antiamoebic activity		
							C	Found	Calcd			I ₅₀ V ₁₀₀ ^a (M n m l %)	Rate ^a (Approx CD ₅₀ mg /kg / day)	Dogs ^a (Approx CD ₅₀ mg /kg / day)
a	H ₂ N	0		80 ^a	177	C ₁₈ H ₁₉ ClN ₂ O	53.70	53.93	4.51	4.67		< 20	425 ^a	
b	(CH ₃) ₂ N	0		40 ^a	206 (d)	C ₁₈ H ₁₉ ClN ₂ O 2HCl	44.38	44.08	4.97	5.18		< 20	> 175	
c	(C ₂ H ₅) ₂ N ^a	2												
d	(C ₂ H ₅) ₂ N ^a	2	A	50 ^a	188 (d)	C ₁₁ H ₁₄ ClN ₂ O 3HCl 1/2H ₂ O	46.38	46.62	6.41	6.43		2.5	> 675 ^a	
e	(CH ₃) ₂ N	3	A	20 ^a	232 (d)	C ₁₈ H ₁₉ ClN ₂ O 3HCl H ₂ O	42.77	42.71	5.98	5.96		5	150 ^a	
f	(C ₂ H ₅) ₂ N	3	A	22 ^a	203 (d)	C ₁₁ H ₁₄ ClN ₂ O 3HCl ^b	47.35	47.55	6.31	6.41		2.5	150	10
	(C ₂ H ₅) ₂ N	3	B	30 ^a	201 (d)	C ₁₁ H ₁₄ ClN ₂ O 2HCl	51.72	51.84	6.64	6.64				
	(C ₂ H ₅) ₂ N	3	A	38 ^a	217 (d)	C ₁₁ H ₁₄ ClN ₂ O 3HCl	47.57	47.70	5.87	5.87		< 200	150	10
	(C ₂ H ₅) ₂ N	3	B	35 ^a	221 (d)	C ₁₁ H ₁₄ ClN ₂ O 2HCl	51.98	52.00	6.16	6.17				
i	(C ₂ H ₅) ₂ N	3	A	32 ^a	223 (d)	C ₁₈ H ₁₉ ClN ₂ O 3HCl	48.77	48.27	6.14	6.26		2.5	75	10
j	(CH ₃) ₂ N	3	A	52 ^a	261 ^a (d)	C ₁₈ H ₁₉ ClN ₂ O 3HCl	47.17	46.78	6.16	6.37		< 200	225 ^a	
k	(CH ₃) ₂ N	4	A	45 ^a	233 (d)	C ₁₈ H ₁₉ ClN ₂ O 3HCl	48.55	48.42	6.56	6.67		< 200	275 ^a	
l	(CH ₃) ₂ N	4	A	45 ^a	208 (d)	C ₁₁ H ₁₄ ClN ₂ O 3HCl H ₂ O	46.87	46.77	6.31	6.39		< 200	275 ^a	
m	(C ₂ H ₅) ₂ N	4 ^a	A	36 ^a	236 (d)	C ₁₈ H ₁₉ ClN ₂ O 3HCl ^a	49.68	49.77	6.80	7.23		10	275	> 10



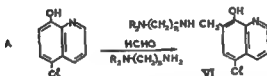
V



VI

disproved the widely accepted theory of antamebic action based upon the release of iodine which all the established 8-hydroxyquinolines contain. However, a lack of satisfactory activity by type (V) compounds against hepatic amoebiasis in hamsters (14) suggested that the side chain might be of such a compound (VI, wt (15), but the low order of ac

further synthesis. Nevertheless, continued efforts of considerable promise. One of them

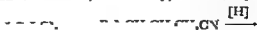


was prepared by the addition of linpropionitrile. The nitrile was nickel to obtain the diamine (B)

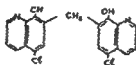


3-(4-methylpiperazinyl) propylamine was similarly prepared from 1-methylpiperazine

4-Diethylaminobutylamine and 4-pyrrolidinobutylamine were prepared by treatment of 3-chlorobutyronitrile with diethylamine and pyrrolidine, respectively,



In general, reaction A proceeded smoothly. A small amount of byproduct 7:7'-methylene-bis-(5-chloro-8 quinolinol) (VII) usually formed in insignificant amount. Previous studies have shown that VII may result from a reversal



VII

of the Mannich reaction (15). The principal difficulty encountered in the

later stages of the preparations, it was found that the products could more easily be isolated by the addition of 2-molar equivalents of anhydrous hydrogen

before formation of the dihydrochloride increased the yields to 45 to 50%. That the desired compounds were obtained in no greater yields is not surprising in view of the fact that primary amines in the Mannich reaction, in contrast to



VIII



IX

through the formation of by-products.

Attempts to synthesize (VI a and b) (Table I) by means of hydrazine and *sym*-dimethylhydrazine in reaction A resulted in failure, with 5-chloro-8-quinolinol having been recovered unchanged. Acetophenone likewise failed to undergo the Mannich reaction with *sym*-dimethylhydrazine. However, a successful Mannich-type condensation with hydrazine has been reported. Frankel and Kliger, obtained *sym*-bis-(2, 2-dinitropropyl)-hydrazine by the reaction of 2, 2-dinitropropanol with hydrazine in glacial acetic acid (16). Hydrazine (VIa) was synthesized quite by accident. In an effort to prepare the dihydrazide

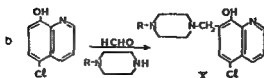
3,5-dinitrobenzoic acid, 2, 2-dinitropropanol and hydrazine (VIa) were synthesized according to reaction D, and the results are summarized in Table II.

TABLE II

5-Chloro 7 (4 substituted 1 *piper* *mylmethyl*) 8 *quinolins*

X	R	Yield, (%)	M.p. (°C)	Formula	Analyses		H Found	Anti amoebic activity	
					Calcd	Found	Calcd	In vitro ^a (Minimal γ/cc)	Raise ^b (Approx CD ₅₀ mg/kg/day)
a.	ClH ₂	50	247 (d) 149	C ₁₆ H ₁₁ ClN ₂ O 2HCl 1/2H ₂ O C ₁₆ H ₁₀ ClN ₂ O	48.21 61.74	48.49 61.12	5.66 6.22	5	>1000
b.	ClH ₂ (CH ₂) ₂	53	275 (d)	C ₁₈ H ₁₃ ClN ₂ O 2HCl	58.72	58.55	7.80	< 200	350
c.	C ₂ H ₅ O ₂ C	77	133 188 (d)	C ₁₇ H ₁₀ ClN ₂ O ₂ C ₁₇ H ₁₀ ClN ₂ O ₂ 2HCl	58.37 48.30	58.34 48.04	5.76 5.23	2.5	>1250
d.	H	82	343 (d) 230 (d)	C ₁₆ H ₁₀ ClN ₂ O 2HCl 2H ₂ O ^a C ₁₆ H ₁₂ ClN ₂ O 2H ₂ O	43.48 53.59	43.47 53.57	5.74 6.42	< 200	> 600
e.	(C ₂ H ₅) ₂ NCO	56	200 (d)	C ₁₈ H ₁₄ ClN ₂ O ₂ 2HCl ^b	49.25	49.16	6.20	< 200	925
f.	H ₃ C ₂ OCH ₂	71	206	C ₁₈ H ₁₄ ClN ₂ O ₂	57.23	57.27	5.40	< 200	350
g.	C ₂ H ₅ SO ₂	100	215 (d) 158	C ₁₈ H ₁₄ ClN ₂ O ₂ S HCl ^c C ₁₈ H ₁₄ ClN ₂ O ₂ S	47.29 51.95	47.24 52.02	5.21 5.45	< 200	725
h.								<2000	

^a Anal. for ionic chlorine. Calcd 18.34. Found 18.30. ^b CH and H₂O analysis corresponds to 3/4 H₂O. Calcd 3.75. Found 3.54. ^c See ref 16 for original synthesis. ^d See footnote r of Table I. ^e See footnote s of Table I.



5-Chloro-7-(1-piperazinylmethyl) 8-quinolol (Xd) was obtained by

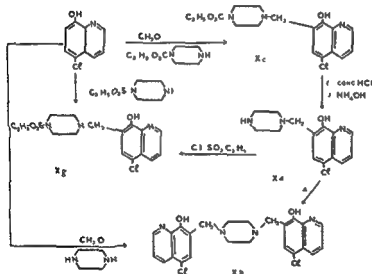


Fig. 1

The compounds of Tables I and II are as follows:

even as the dihydrochloride salts

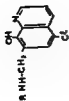


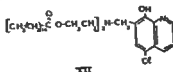
TABLE III

5-Chloro-7 (alkylamino)quinolines

XI	R	Proc- dure	Yield (%)	Mp °C	Formula	Analyses		Found	H ^a	Antiamoebic activity	
						Calcd	Found			In 1 trial ^b (Mineral y/cc)	Rate ^c (Approx CD ₅₀ /mg/kg day)
a	CH ₃ (CH ₂) ₃ CH C ₆ H ₅	A	43 ^a	209 (d)	C ₂₄ H ₂₀ ClN ₂ O·2HCl	54.90	55.28	6.91	7.14	6.3	375
b	CH ₃ (CH ₂) ₆	A	53 ^a	197 (d)	C ₂₇ H ₂₂ ClN ₂ O·2HCl	53.76	53.92	6.64	6.64	<200	625
c	CH ₃ (CH ₂) ₇ ^a	A	41 ^b	190 (d)	C ₂₈ H ₂₄ ClN ₂ O·2HCl	54.90	55.21	6.91	7.09	<200	>300

^a Yellow solid from ethanol-methanol^b Yellow solid from absolute ethanol^c Intermediary octylamine made from octyl bromide and potas-sium phthalimide by use of the general procedure of Sheehan, J. C. and Bollhofer, W. A. (1950) *J. Amer. Chem. Soc.*, 72, 2786^d See foot note 1, Table II

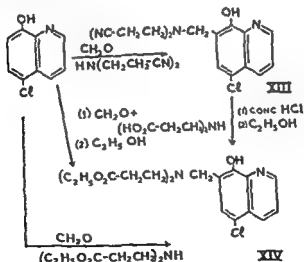
A few other compounds which may not be conveniently listed in Tables I, II or III were synthesized. 5-Chloro-7-*bis*-(2-hydroxyethyl)-aminomethyl]-8-quinolinol dipalmitate hydrochloride (XII) was obtained by the action of palmitoyl chloride upon 5-chloro-7-*bis*-(2-hydroxyethyl)-aminomethyl]-8-



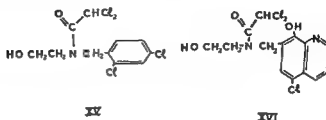
quinolinol. Like the agents of Table III, it was virtually insoluble in water.

When 5-chloro-8-quinolinol, paraformaldehyde and $\beta\beta$ -iminodipropionitrile were allowed to heat at reflux temperature in alcohol, the expected 5-chloro-7-*bis*-(2-cyanoethyl-aminomethyl)-8-quinolinol (XIII) was obtained (Fig 2). Treatment of (XIII) with boiling alkali failed to yield the corresponding diacid but instead gave 7,7-methylene-*bis*-(5-chloro-8-quinolinol) (VII), thus representing another example of the reversal of the Mannich reaction under

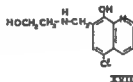
isolated in both cases. The product thus obtained in three different ways was assigned structure (XIV). It was somewhat surprising to find that complete esterification had occurred during the process of recrystallization of the diacid hydrochloride from alcohol.



Upon learning the interesting anti amoebic activity of N (2,4 dichlorobenzyl)-N-(2-hydroxyethyl) dichloroacetamide (XV) in animals (21), we decided to attempt the synthesis of N (5 chloro 8-hydroxy 7 quinolylmethyl) N (2 hydroxyethyl) dichloroacetamide (XVI). Intermediate 2 (5-chloro 8 hydroxyquino-lylaminomethylamino) ethanol (XVII) was prepared by use of ethanolamine in reaction D. (XVII) was then allowed to react with methyl dichloroacetate to



give (XVI). Difficulty was encountered in this reaction owing to the low solubility of (XVII) in common organic solvents. Reaction was finally effected by allowing a suspension of (XVII) in excess methyl dichloroacetate to stand for several weeks.



Pharmacological results. The anti-amoebic evaluation of these compounds was oriented around the primary goal of developing useful drugs rather than a

substances were to be tested against hepatic amoebiasis in hamsters. As past experience had suggested that greater emphasis be placed on *in vivo* performance than on *in vitro* potency, the endpoint of activity *in vitro* was determined for less than half of the compounds. It also is to be emphasized that the effects of most of the compounds *in vivo* are based on relatively small numbers of animals and at best represent only rough approximations.

Data on the anti amoebic activities are given together with a summary of the results in Tables I, II and III. Anti amoebic data on Table IV

I, were deemed worthy of trial in dogs

1)—had in rats a therapeutic index (ratio

of minimum effective to maximum tolerated dose) of two or more, and three of them cured infections in dogs without any gross evidence of toxicity. It is to be noted that compounds with three methylene groups appeared to be superior to this with either two or four to six methylene groups.

TABLE IV
Miscellaneous basic 5-chloro 8-quinolins

Compound	Antiamoebic activity	
	in <i>strep</i> (Minimal γ cc)	Rais ^b (Approx CD ₅₀ mg/kg/day)
XII	~2000	>2150
XIV	~200	375
XVI	20	>175
XVII	25	600

^a See foot note *r* of Table I
^b See foot note *s* of Table I

Compound (VII) of Table I [5-chloro 7-(3-diethylaminopropylaminomethyl)-8-quinolinol] appeared from several standpoints to be one of the most effective substances against experimental intestinal amoebiasis in rats and dogs. It also proved to be as active as chloroquine (b) when tested orally against hepatic amoebiasis in hamsters. These findings resulted in the decision to conduct a pre-clinical toxicity study of this substance, and the latter work has sustained interest in the compound as a possible anti-amoebic drug.

SUMMARY

A continuation of the search for antiprotozoan agents among phenolic quinolinol bases has led to the synthesis of a group of such bases from 5-chloro-8-quinolinol. Several have been found to be highly effective against *Entamoeba histolytica* *in vitro* and against experimental intestinal amoebiasis in rats and dogs. One of them, 5-chloro-7-(3-diethylaminopropylaminomethyl)-8-quinolinol (K-322), has been studied toxicologically and is now being investigated for anti-amoebic activity *in man*.

REFERENCES

- 1 See reference no. 1 p 481
- 2 See reference no. 2 p 481
- 3 Burckhalter, J. H., Tenick, E. H., Jones, F. M., Jones, P. A., Holcomb, W. I. and Rawlins, A. I. (1918) *J. Amer. Chem. Soc.* 70: 1363
- 4 Hockenga, M. T. (1918) *J. Hyg. Soc. Lond.* 31: 139
- 5 Thompson, P. F. (1951) *Amer. J. Trop. Med.* 3: 250
- 6(a) Thompson, P. F. (1918) *J. Hyg. Soc. Lond.* 31: 139
- 6(b) Gurd, G. W., Wilson, A. J. and Reimann, J. H. (1951) *Amer. J. Trop. Med.* 3: 707
- 7 Burckhalter, J. H. and Wilson, W. L. (unpublished work)
- 8(a) Thompson, P. F. and Wilson, W. L. (unpublished work)
- 8(b) Thompson, P. F. (1951) *Amer. J. Trop. Med.* 3: 450
- 9 Burckhalter, J. H., Tenick, E. H., Jones, F. M., Jones, P. A., Holcomb, W. I. and Rawlins, A. I. (1918) *J. Amer. Chem. Soc.* 70: 1363
- 10 Edwards, J. W. (1911) *J. Surg. Gynecol. Obstet.* 6: 987
- 11 Thompson, P. F., Reimann, J. H., McCarthy, D. A., Bayles, F. J. (1941-43), Vol. I
- 12 Teddi, J. & Chermak, S. (1953) *The Pharmacological Basis of Therapeutics*, Vol. 2 p 1216
- 13 Gaskman, J. S. and Gillman, A. (1955) *The Pharmacological Basis of Therapeutics*, Vol. 2 p 1216
- 14 MacMillan, C. A. (1955) *The Pharmacological Basis of Therapeutics*, Vol. 2 p 1216
- 15 Burckhalter, J. H., and Edgerly, W. F. (1951) *J. Amer. Chem. Soc.* 73: 4837

ANTIAMOEBIAC ACTION OF SUBSTITUTED QUINOLINES, QUINALDINES, QUINAZOLINES, QUINAZOLONES, CHROMANONES, THIOCHROMANONES, DIAMINOALKANES, BENZYLAMINES AND CRESOLS

■ S KAUSHIK

From the Central Drug Research Institute Lucknow

This paper describes the testing of various compounds (1-8) for activity against *Entamoeba histolytica* with *Entamoeba histolytica* strains, belonging to various clinical value have also been included for their usefulness in evaluating the methods and criteria employed and in elucidating the structure-activity relationships. Emetine, conessine, chiniofon and vioform served as reference drugs and their activity was almost in agreement with the findings of other workers.

MATERIALS AND METHODS

In vitro tests The EA strain of *E. histolytica* obtained from Dr R A Neal, Wellcome Laboratories of Tropical Medicine, London, served as the test organism. The general test procedure was similar to that described in earlier papers (1-4).

The compounds were brought into solution or into emulsion with the help of organic solvents and

strain of *E. histolytica* was used. The test was carried out in a similar manner to that described in earlier papers (1-4). The results are given in Table I. The compounds were tested at a concentration of 2.02 (range 1-1) and 2.06 (range 1-1) for abundance of amoebae and faecal lesions respectively.

The experimental animals were albino rats of both sexes (25 ± 5 g) maintained on stock diet of the C D R I. Each rat was inoculated intracaecally with 0.5 cc of the inoculum (i.e., 50,000–100,000 amoebae). The chemotherapeutic agents were administered 24 hr after the operation and the effect was investigated by oral administration of the drug for six consecutive days. The

after seven days and the extent of infection was assessed on the basis of caecal lesions, the appearance of faecal contents, and presence and abundance of amoebae in caecal mucosa. The state of the caecum of each rat was assessed by an arbitrary scoring on the basis of the following scheme:

Caecal lesions (0 = apparently normal caecum, 1 = questionable or slight thickening, 2 = moderate or restricted thickening, 3 = marked thickening possibly with slight ulceration, 4 = great thickening with much ulceration).

Abundance of amoebae (0 = none, 1 = very few (<1 in 20 fields), 2 = few (1–5 per 20 fields), 3 = moderately numerous (1–5 per field), 4 = numerous (>10 per field)).

Appearance of faecal contents (0 = apparently normal, 4 = mucus and pus present in small quantities).

order

3 = 11

order of activity

Sources of test compounds: Most of the compounds examined have been prepared at the Central Drug Research Institute (1–3) (RG, I, & BP series) and others have been received from Calcutta (4) BPC series, Lucknow (5, 6) (YDK and SLA series) and Vikram University (7, 8) (CNK series). The known compounds were purchased from commercial sources.

RESULTS AND DISCUSSION

In the present study, *in vitro* amoebicidal activity was found to be widely distributed among the various chemical series. Thus 12 of 115 compounds were amoebicidal at concentration of 500–1000 $\mu\text{g/cc}$, 35 at 100–500 $\mu\text{g/cc}$, 43 at 10–100 $\mu\text{g/cc}$ and 25 at <10 $\mu\text{g/cc}$. Out of 68 compounds that were active *in vitro* at 10–100 $\mu\text{g/cc}$ or less, 35 were tested against intestinal infection in rats and 11 of these proved effective in rats in varying degrees. In general, the correlation between *in vitro* and *in vivo* testing as revealed by a study of 53 compounds which were tested by both methods was poor, particularly in miscellaneous amines (Table VI) and chromanones (Table X). In most of the cases the compounds which were quite active *in vitro* were later found to be inactive *in vivo*. However, compounds I_{10} and I_{19} showed comparatively feeble *in vitro* activity but were slightly to moderately effective *in vivo*. The *in vitro* tests provide a direct assessment of amoebicidal action of the compounds and yield useful information on the relation between structure and activity whereas the *in vivo* tests are valuable for assessing therapeutic possibilities.

Quinaldines: In Table I, the *in vitro* activity of all the compounds is fairly high excepting RG6 and 7. Perhaps the introduction of large branched groups

RG2 is not toxic and not active whereas RG11 is very toxic but not so very active

p Chlorophenylamino substitution at 4 position in the quinolines yields compounds of promising activity (RG 1 and RG 12). The hydroxy derivative (RG 12) however, shows outstanding activity and has been selected for further studies.

Quinolines The *in vitro* activity is not effected by varying the length of alkyl chain (YDK 1-4). In this series (Table II) perhaps the presence of polar sulphonic group has a damping effect on the *in vitro* activity of quinolines (YDK 1-6) because this group usually results in unfavourable oil water partition coefficient, but slight *in vitro* activity is exhibited by YDK 5 and YDK 6 in which methylpiperidino and morpholino substitutions have been made at 7 position. However, both the compounds at 100 mg/kg dose were inactive in rats. In our experiments chinofon (yatren manufactured by Bayer in Germany) shows slightly higher activity than crystallized viofilm at 300 mg/kg which may probably be due to experimental variations. RG 15 which resembles viofilm except that there is a $-\text{OCH}_3$ group at 8 position instead of $-\text{OH}$ group is quite active *in vitro* and *in vivo*.

TABLE I
6 Methoxy 4 substituted amino 8 hydroxy (and methoxy) quinolines
Screening test results against *G. histolytica* *in vitro* and in rats

COMPOUND NO	SUBSTITUENTS	R	DOSE mg/kg	ORDER OF ACT V TV	IN VITRO MIN. CONC. AMEBIC INH. CONC. $\mu\text{g}/\text{ml}$
RG 4	C_6H_5	OCH_3	100	0	6
RG 5	$n\text{-C}_7\text{H}_{15}$	OCH_3	100	0	8
RG 6	$\text{SCH}_2(\text{CH}_2)_2\text{N}(\text{C}_2\text{H}_5)_2$	OCH_3	>160	00	8
RG 7	$(\text{CH}_3)_2\text{N}(\text{C}_2\text{H}_5)_2$	OCH_3	>260	100	1
RG 8	SCH_2OCH_3	OCH_3	>175	100	125
RG 9	SCH_2Cl	OCH_3	>160	100	125
RG 10	C_6H_5	OCH_3	>100	100	8
RG 11	$n\text{-C}_8\text{H}_{17}$	OH	30-100	3-4	4
RG 12	$\text{C}_6\text{H}_4\text{OH}$	OH	>175	2-3	10-100
RG 13	$\text{CH}_2\text{-C}_6\text{H}_5$	OH	>200	0	4
RG 14	$\text{CH}_2\text{-C}_6\text{H}_4\text{OH}$	OH	>200	0	8
RG 15	$\text{CH}_2\text{-C}_6\text{H}_4\text{OCH}_3$	OH	100	0	16
RG 16	$\text{CH}_2\text{-C}_6\text{H}_4\text{Cl}$	OH	25	2	2-4
RG 17	$\text{CH}_2\text{-C}_6\text{H}_4\text{OCH}_3$	OH	>200	4	2-4



Quinolones All the derivatives (Table III) show feeble *in vitro* activity except 182 in which benzyl has been substituted at 3 position and which is sufficiently active. The compound 182 is highly active *in vivo* for the size of dose, compound 174 is also very active in rats but is rather toxic.



Quinazolines In this series (Table IV) the *in vitro* activity is generally less than in the variously substituted quinazolones. However, compounds I₃₂ and I₃₃ show fair activity *in vitro* and compounds I₁₀, I₁₉ and I₃₅ show mediocre *in vivo* activity.

Gresols Among the analogues of camoform (Table V) SLA 16, 19 and 22 are more active *in vitro* than SLA 18, 21 and 23. Apparently the activity is sharply decreased as a result of tertiary butyl substitution. It would, however, be interesting to compare the activity of tertiary butyl substituted compounds with normal butyl compounds. The piperidino and morpholino substitution makes little difference in the activity.

TABLE II

5, 7 Di substituted 8 hydroxy (and methoxy) quinolines
 Screening test results against *E. histolytica* *in vitro* and *in rats*



COMP OUND NO.	SUBSTITUENTS			MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MAXIMUM AMO- FICIDAL CONC μg/ml
	X	Y	R				
YDK 1	SO ₃ H	CH ₂ N (CH ₃) ₂	OH	>200	-	-	1000
YDK 2	SO ₃ H	CH ₂ N (C ₂ H ₅) ₂	OH	>200	-	-	500-1000
YDK 3	SO ₃ H	CH ₂ N (C ₃ H ₇) ₂	OH	>140	-	-	1000
YDK 4	SO ₃ H	CH ₂ N (C ₂ H ₄ OH) ₂	OH	>125	-	-	1000
YDK 5	SO ₃ H	CH ₂ N 	OH	>200	150 100	2 0	250
YDK 6	SO ₃ H	-CH ₂ N 	OH	>200	100	1	250
YATRO	SO ₃ Na	1	OH	-	300	5	125
YKFORM	Cl	1	OH	-	300	3	10
RG 15	Cl	1	OCH ₃	>300	300	5	10

Miscellaneous amines The various amines and diamines synthesized on the basis of partial structure of emetine (BPC and CNK series, (Table VI) give high *in vitro* activity but are ineffective in rats. In the 1 substituted benzylamino-2 piperidino (and morpholino) ethanes (Table VII) the piperidino and morpholino substitution does not make any appreciable difference in their *in vitro* activity. The *in vitro* activity is still less in the various 1 substituted benzylamino-3 piperidino (and morpholino) propanes (Table VIII) but morpholino derivatives exhibit higher activity than piperidino substituents in this series. None of the compounds

belonging to 1-piperidino 5 substituted aminopentanes (Table IX) evinced sufficient *in vitro* activity but compounds BP 6 BP 8 and BP 9 are slightly more active than the remaining ones. This observation may tend to indicate that in this class of compounds the presence of 3 4-dialkyl (or phenyl) substituents in the molecule may be important for activity. However, *in vivo* activity in BP 8 is totally absent.

TABLE III
3-Substituted 8 hydroxy (and methoxy) quinoxalones
Screening test results against *L. histolytica* *in vitro* and in rats

COMPOUND NO	SUBSTITUENTS		MIL MIN TOLERANCE (mg/kg)	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO PERCENT INHIBITION AT CONC. 1:1000
	X	R				
1	H	H	0CH ₃	> 150	-	-
2	CH ₃		0CH ₃	> 240	-	100 - 123
3	C ₂ H ₅		0CH ₃	> 240	100	
4	B-C ₂ H ₅		0CH ₃	> 33	33	2
5	B-C ₂ H ₅		0CH ₃	> 100	100	1
6	CH ₃		0CH ₃	> 130	50	1
7	CH ₃		OH	> 220		
8	C ₂ H ₅		OH	> 100	100	2
9	B-C ₂ H ₅		OH	> 45	33	814
10	B-C ₂ H ₅		OH	> 200	100	1
11	CH ₃		OH	> 100	33	110



Chromanones and thiochromanones. The thiochromanones are found to be more active *in vitro* than the corresponding chromanones (Table X) and it would appear that thiochromanone nucleus itself may be a more desirable pharmacophore for the synthesis of amoebicidal compounds. But the high *in vitro* activity in both the series bears little relationship to the *in vivo* activity which is low YDK 10, 11, 14 and SLA 11 and 12 (chromanones) are inactive in rats and similarly among thiochromanones YDK 20 and SLA 2 are inactive but YDK 21 has slight activity. The activity in compounds of chromanone series varies from group to group as follows: 6-Cl and 6-Cl are more active than 6-Cl and 8-Cl are more active than 6-Cl, 1 and 8-Cl and 8-Cl show almost similar activity and the piperidino substitution is slightly less active than the morpholino substituents. In the thiochromanone series 6-OCH₃ and 6-Cl or 8-OCH₃ and 8-Cl do not show similar activity and no conclusive remarks can be made due to insufficient number of corresponding pairs in the series. Similarly no decisive comments can be made

TABLE IV

4 Substituted amino 8 methoxy quinazolines Screening test results against *E. histolytica* in vitro and in rats



COMPOUND NO	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO M.N.M.U. AMEBICIDAL CONC. $\mu\text{g}/\text{ml}$
118	$\text{p-C}_6\text{H}_5$	> 100	100	2	1000
132	$\text{p-C}_5\text{H}_{11}$	> 50	10	0	63
138	$\text{iso-C}_5\text{H}_{11}$	> 70	33	2	51-63
119	$\text{m-C}_6\text{H}_{13}$	> 200	100	3	1000
126		> 60	50	0	500
127		> 100	100	1	1000
131		200	100	0	1000
129		> 100	100	0	250-300

TABLE V

2 Allyl 4 substituted 6 morpholino methyl phenols Screening test results against *E. histolytica* in vitro and in rats



COMPOUND NO	SUBSTITUENTS		MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO M.N.M.U. AMEBICIDAL CONC. $\mu\text{g}/\text{ml}$
	X	R				
SLA 16	CH_3		>100	100	1	63
SLA 17	Cl		>100	00	2	63
SLA 18	C_6H_5		>100			250
SLA 19	CH_3		>100	00	1	63
SLA 20	Cl		>100	100	0	16-31
SLA 21	C_6H_5		>100		-	1000
SLA 22	CH_3		>100	100	0	31
SLA 23	C_6H_5		>100	-	-	250
CAMO-FORM			>100	100	2	53-125

whether alkyl substitution renders the compounds less active in comparison to the corresponding halogen substituted compounds or not

In the light of above observations it may be useful to make some comments regarding the mechanism of antiamoebic action among the compounds of quinoline ring system. Some of the compounds included in this study have been tested in the Antibiotic Section of the Institute against a variety of bacteria and in preliminary screening are found to be inactive against them at 1:5000 dilution. But

TABLE VI

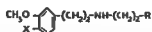
Miscellaneous amines

Screening test results against E. histolytica in vitro and in rats

SERIES A		SERIES B		SERIES C		SERIES D	
COMPOUND NO	SUBSTITUENTS		MINIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	M.V. TO D. INDEXICAL CONC. mg/ml	
	Y	R					
SERIES A							
SAC 3		D-C ₃ H ₇	> 100	100	1	8.14	
SERIES B							
CNE 1		C ₂ H ₅	> 75	-		12.5	
CNE 2		n-C ₃ H ₇	> 100			6.5	
CNE 3		D-C ₄ H ₉	> 100	100	1	21-6.5	
CNE 4		D-C ₅ H ₁₁	> 100			16	
SERIES C							
SAC 3	-	D-C ₄ H ₉	> 100	100	1	6	
SERIES D							
SAC 3	3	D-C ₆ H ₁₃	> 33	33	0	6	
CNE 5	3	-CH ₃	> 100			16	
CNE 6	3	C ₂ H ₅	> 100			16	
CNE 7	3	D-C ₃ H ₇	> 100	100	1(7)	16	
CNE 8	4	D-C ₃ H ₇	> 100	100	1(7)	8	
CNE 9	4	n-C ₄ H ₉	> 100	100	0	4.8	

TABLE VII

1-Substituted benzylamino 2-piperidino (and morpholino) ethanes
Screening test results against *E. histolytica* in vitro



COMP- OUND NO	SUBSTITUENTS		MAXIMUM TOLERA- TED DOSE mg/kg	IN VITRO MIN- IMUM ANOEBCI- DAL CONC μg/ml
	X	R		
SLA 25	-OCH ₃	-N ₅	> 100	63-100
SLA 26	-OCH ₃	-N ₆	> 50	63-100
SLA 24	OC ₆ H ₅	-N ₅	> 100	100-125
SLA 28	OC ₆ H ₅	-N ₆	> 100	100
SLA 29	OC ₂ H ₁₁	-N ₅	> 100	125
SLA 27	OC ₂ H ₁₁	-N ₆	> 70	500
SLA 30	OC ₆ H ₅	-N ₆	> 100	100-125

TABLE VIII

1-Substituted benzylamino-3-piperidino (and morpholino) propanes
Screening test results against *E. histolytica* in vitro







COMP- OUND NO	SUBSTITUENTS		MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIM- UM ANOEBCID AL CONC μg/ml
	X	R		
YDK 25	COCHCl ₃ N-CH ₂ -C ₆ H ₃ (Cl) ₂	-N ₅	> 100	250
YDK 26	COCHCl ₂ N-CH ₂ -C ₆ H ₃ (Cl) ₂	-N ₆	> 100	1000
YDK 27	-NN-CH ₂ -C ₆ H ₃ (Cl) ₂	-N ₅	> 100	250
YDK 28	-NN-CH ₂ -C ₆ H ₃ (Cl) ₂	-N ₆	> 100	250-500
YDK 29	-NN-CH ₂ -C ₆ H ₃ (OCH ₃) ₂	-N ₅	> 100	125
YDK 30	-NN-CH ₂ -C ₆ H ₃ (OCH ₃) ₂	-N ₆	> 100	500

being the most active compounds. In 4 substituted 8 methoxy quinazolines absence of activity may be due to lack of chelation but the activity in I₃₃ and

TABLE IX

1 Piperidino 5 substituted aminopentanes
Screening test results against *E. histolytica* in vitro



COMPOUND NO	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIMUM AMOEBIICIDAL CONC. μg/ml
BP1(202)	NH CH ₃	> 125	100
BP2(204)	NH-C ₂ H ₅	> 150	100
BP4(205)	NH CH(CH ₃) ₂	> 150	100
BP5(206)	NH C ₆ H ₅	> 140	100
BP6(210)	NH CH ₂ - 	> 150	100
BP7(208)	NH-CH ₂ - 	> 125	100
BP8(209)	NH CH ₂ - 	> 150	100 (10-2 mg 8 days O) ^a
BP9(211)	NH H ₂ C- 	> 125	100
BP3(212)	N(CH ₃) ₂	> 130	100

^aIndicates that four rats were treated at 150 mg/kg for 6 days and showed an order of activity equal to 0

TABLE X

Substituted (1) chromanones and (2) thiochromanones
Screening test results against *E. histolytica* in vitro



COMPOUND NO	SUBSTITUENTS		MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MICROMICROBIAL CONC. μ g/ml
	X	Y	R	
YDK 7	6-Cl		N(CH ₃) ₂	31
YDK 8	6-Cl		N	3
YDK 9	6-Cl		N	63
YDK 16	6-Cl		NHCH ₃	200
SLA 14	6-Cl		N(CH ₃) ₂	20
SLA 11	6-Cl		N	100
SLA 13	6-Cl		N	—
SLA 15	6-Cl		NHCH ₃	100
YDK 10	8-Cl		N(CH ₃) ₂	175
YDK 11	8-Cl		N	120
YDK 12	8-Cl		N	100
SLA 12	8-Cl		N	130
SLA 1	8-OCH ₃		N	—
SLA 3	8-OCH ₃		N	—
YDK 13	6,8-Cl		N(CH ₃) ₂	200
YDK 14	6,8-Cl		N	140
YDK 15	6,8-Cl		N	170

(B)



YDK 17	6-Cl	N(CH ₃) ₂	> 100	8
YDK 18	6-Cl	N	> 160	16 — 31
YDK 19	6-Cl	N	> 160	16
SLA 16(a)	6 OCH ₃	N	—	(100 mg/kg/6 days) *
YDK 20	8-Cl	N(CH ₃) ₂	> 150	(100 mg/kg/6 days) *
YDK 21	8-Cl	N	> 130	(100 mg/kg/6 days) *
YDK 22	8-Cl	N	> 150	4
YDK 23	8-H	N	> 150	8
YDK 24	8-H	N	> 200	8 — 16
SLA 5	8-CH ₃	N	> 100	31
SLA 7	8-CH ₃	N(CH ₃) ₂	> 220	16
SLA 9	8-CH ₃	NHCH ₃	—	8 — 16
SLA 2	8-OCH ₃	N	> 100	(100 mg/kg/6 days) *
SLA 4	8-OCH ₃	N	—	31
SLA 6	8-C ₂ H ₅	N	—	16
SLA 8	8-C ₂ H ₅	N(CH ₃) ₂	—	6 — 31
SLA 10	8-C ₂ H ₅	NHCH ₃	> 100	16 — 31

* SAME AS IN FOOTNOTE TO TABLE X

SUMMARY

The amoebicidal activity of 115 substituted quinolines, quinazolidines, quinazolines, quinazolones, chromanones, thiochromanones, diaminoalkanes, benzylamines and cresols has been tested against *P. histolytica* *in vitro* and 53 compounds were tested in intestinal amoebiasis in rats

Arr
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quinazolid
studies
activity both *in vitro* and *in vivo*

In the quinazolone series (Table III) compounds I₃₆ and I₄₃ and in quinazoline series (Table IV) compounds I₁₀, I₁₉, and I₂₁ are fairly active

6 Morpholino methyl 2 allyl-4 methylphenol (SLA 16) and 6 morpholino methyl 2 allyl-4 chlorophenol (SLA 17) which have been prepared as analogs of camoform show activity comparable to camoform. In this series, tertiary butyl substitution, however, decreases the activity sharply

Some of the amines and diamines synthesized on the basis of partial structure of emetine show considerable *in vitro* activity but they are less active *in vivo* (Table

substitution

The thiochromanones are more active than the corresponding chromanones (Table X) but the high *in vitro* activity found in these series is not reflected in the experimental amoebiasis in rats

ACKNOWLEDGMENTS

The author is highly grateful to Dr F Hawling, Colombo Plan Expert, for stimulating criticism and continued interest in the work. Thanks are also due to Drs H Mukerji, M L Dhar and B N Singh for advice and encouragement to Drs B Pathak, C N Kachru, Y D Kulkarni, R N Iyer, S L Arora and R Gopalachari for the supply of drug samples and to Mr A K Sarkar and Mr V K Misra for technical assistance

- 15 Balamuth W (1946) *Ame J Clin Path* **16** 380
 16 Jones W R (1946) *Ann Trop Med Parasit* **40**: 380

J Trop Med Hyg **4** 224

POTENTIAL AMOEBOCIDES PART VIII SYNTHESIS OF MANNICH
BASES OF 4, 5, 6-, AND 7 HYDROXYQUINOLINES, 4 HYDROXYQUI-
NAL DINE, AND 1 2 3 4 TETRAHYDRO 6 HYDROXYQUINOLINE

J S TANDON R N IYER AND R GOPALACHARI

From the Central Drug Research Institute Lucknow

teability *per se* is obviously not necessary for such deactivation for otherwise one
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of

quinoline have now been synthesized and are reported in this present communi-
cation. In all these compounds the relative positions and the spatial disposition
of the side chain nitrogen functions and of the phenolic residues are such as to
permit facile chelation.

It may be pointed out that with the extra basic centre in the molecule these
new compounds may tend to facilitate the formation of chelate complexes at a
pH slightly higher than 7 of Phillips *et al* (2). This would constitute an
advantage over the pH of the lower intestinal contents (and of the biological
fluids) which may be above 7. Thompson
methyl-8-hydroxyquinoline
than 5, 7-diiodo-8-hydroxy-
ity may be an expression of
the facile chelation of this compound in the lower regions of the intestine because
of its higher basicity. Since however, this compound is derived from 8-hydroxy-
quinoline its use as a systemic amoebicide is likely to be limited due to its
deactivation by the intact red cell wall.

Bueckhelter *et al* (4) have reported the synthesis of 7-diethylamino (and
piperidino) methyl 8-hydroxyquinolines and of 8-diethylaminomethyl 7-hydroxy-
quinoline by a procedure involving the heating of an equimolecular mixture
of the hydroxy compound, formaldehyde and the amine on the water bath.
Following their procedure we were unable to obtain our compounds in a pure
form. The products obtained in this working appeared to be mixtures from
which only the corresponding bisquinolylmethanes could be isolated in a pure
form. If, however, the reaction is carried out at the room temperature or even
slightly lower than that the required products are obtained in good yields.

5 Hydroxyquinoline was prepared by the reversed Bucherer reaction from 5 aminoquinoline (5). Experimental details of this reaction are included in the paper being published elsewhere.

tion, in a 50% yield

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hydroxyquinoline with hydrogen in the presence of Raney nickel (W4) (8) at a pressure of 50 atmosphere

The Mannich bases of 5, 6 and 7 hydroxyquinolines are generally low melting solids or viscous liquids. They are unstable and decompose slowly into the corresponding bisquinolyimethanes. They form sharp melting picrates which are soluble in chloroform, benzene and petrol ether in that order. The Mannich bases of 4 hydroxyquinoline and 4 hydroxyquinoline however are more stable. The structures of the Mannich bases obtained from 4 hydroxyquinoline are considered by analogy of Price and Jackson (9) to be the normal 3 substituted compounds instead of 2 β aminoethyl derivatives.

The compounds now reported are under investigation for their amoebicidal activity in the Division of Microbiology of this Institute. The results will be communicated later.

EXPERIMENTAL

5 Hydroxyquinoline 5 Aminoquinoline (obtained by the reduction of 10 g of 5 nitroquinoline (10) was refluxed with sodium metabisulphite (98% pure, 60 g) in water (80 cc) for 20 hr. The reaction mixture was cooled and treated with aqueous sodium hydroxide (10%) to pH 10 and extracted with benzene to remove the unreacted amine. The clear yellow aqueous solution was heated

TABLE I

Mannich bases from 4 hydroxyquinoline



S NOS	R	MOLECULAR FORMULA	M P O _c HYDROCHLORIDE	FOUND	REQUIRED
				N	H
250	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_5 \\ \searrow \text{C}_6\text{H}_5 \end{matrix}$	$\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}$	168 (d)	9.4	9.8
251	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_4 \\ \searrow \end{matrix}$	$\text{C}_7\text{H}_8\text{N}_2\text{O}$	205 (d)	11.3	11.6
252	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_3\text{O} \\ \searrow \end{matrix}$	$\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$	160 (d)	11.4	11.5

* MELTING POINTS WERE DETERMINED IN SEALED CAPILLARIES AS THESE COMPOUNDS WERE FOUND TO BE VERY HYGROSCOPIC

TABLE II

Mannich bases from 4 hydroxyquinoline

S NOS	R	MOLECULAR FORMULA	M P O C	FOUND			REQUIRED		
				C	H	N	C	H	N
253	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_2\text{H}_5 \\ \searrow \text{C}_2\text{H}_5 \end{matrix}$	$\text{C}_9\text{H}_{20}\text{N}_2\text{O}$	324	74.0	7.8	11.5	73.8	8.2	11.5
254	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_4\text{H}_9 \\ \searrow \text{C}_2\text{H}_5 \end{matrix}$	$\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}$	310	76.3	9.8	9.7	76.0	9.3	9.3
255	$-\text{CH}_2-\text{N} \begin{matrix} \diagup \text{C}_6\text{H}_4 \diagdown \end{matrix}$	$\text{C}_{10}\text{H}_{20}\text{N}_2\text{O}$	302	74.5	8.0	10.6	75.0	7.8	10.9
256	$-\text{CH}_2-\text{N} \begin{matrix} \diagup \text{C}_6\text{H}_3\text{O} \diagdown \end{matrix}$	$\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_2$	320	70.0	7.2	10.8	69.8	7.0	10.8

TABLE III

Mannich bases from 5 hydroxyquinolines

S NOS	R	R ₂	MOLECULAR FORMULA	M P O C	FOUND			REQUIRED		
					C	H	N	C	H	N
257	$\text{CH}_3-\text{N} \begin{matrix} \nearrow \text{C}_2\text{H}_5 \\ \searrow \text{C}_2\text{H}_5 \end{matrix}$	H	$\text{C}_8\text{H}_{16}\text{N}_2\text{O}$	VISCOUS OIL PICRATE 122	72.9	8.4	12.6	73.0	7.8	12.2
258	$-\text{CH}_2-\text{N} \begin{matrix} \diagup \text{C}_6\text{H}_4 \diagdown \end{matrix}$	H	$\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}$	OIL PICRATE 179	74.6	8.4	11.8	74.4	7.4	11.6
259	$-\text{CH}_2-\text{N} \begin{matrix} \diagup \text{C}_6\text{H}_3\text{O} \diagdown \end{matrix}$	$-\text{CH}_2-\text{N} \begin{matrix} \diagup \text{C}_6\text{H}_3\text{O} \diagdown \end{matrix}$	$\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_2$	169	66.3	7.2	12.0	66.5	7.3	12.2

5-Hydroxyquinoline was prepared by the reversed Bucherer reaction from 5-aminoquinoline (5). Experimental details of this reaction are included in this paper because the details of the procedure are not available in the literature. 6- and 7-Hydroxyquinolines were obtained from *para* and *meta* anilines, respectively, by Skraup synthesis as modified by Šířukov (6), followed by demethylation, in a 50% yield.

to

h₂

yield either by this procedure or by the use of sodium and alcohol or tin and hydrochloric acid. It was finally obtained in 95% yield by the reduction of hydroxyquinoline with hydrogen in the presence of Raney nickel (W4) (8) at a pressure of 50 atmosphere.

The Mannich bases of 5, 6 and 7-hydroxyquinolines are generally low melting solids or viscous liquids. They are unstable and decompose slowly into the corresponding bisquinolylnethanes. They form sharp melting picrates which are soluble in chloroform, benzene, and petrol ether in that order. The Mannich bases of 4-hydroxyquinoline and 4-hydroxyquinoline, however, are more stable. The structures of the Mannich bases obtained from 4-hydroxyquinoline are considered by analogy of Price and Jackson (9), to be the normal 3-substituted compounds instead of 2-β-aminoethyl derivatives.

The compounds now reported are under investigation for their amoebicidal activity in the Division of Microbiology of this Institute. The results will be communicated later.

EXPERIMENTAL

5-Hydroxyquinoline. 5-Aminoquinoline (obtained by the reduction of 10 g of 5-nitroquinoline (10) was refluxed with sodium metabisulphite (98% pure, 60 g) in water (80 cc) for 20 hr. The reaction mixture was cooled and treated with aqueous sodium hydroxide (10%) to pH 10 and extracted with benzene to remove the unreacted amine. The clear yellow aqueous solution was heated

TABLE I

Mannich bases from 4-hydroxyquinoline



S NOS	R	MOLECULAR FORMULA	M P O _c HYDROCHLORIDE	FOUND	REQUIRED
				N	H
250	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_5 \\ \searrow \text{C}_6\text{H}_5 \end{matrix}$	$\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}$	168 (d)	9.4	9.8
251	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_4 \\ \searrow \end{matrix}$	$\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}$	205 (d)	11.3	11.6
252	$-\text{CH}_2-\text{N} \begin{matrix} \nearrow \text{C}_6\text{H}_3\text{O} \\ \searrow \end{matrix}$	$\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2$	160 (d)	11.4	11.5

* MELT NG POINTS WERE DETERMINED IN SEALED CAPILLARIES AS THESE COMPOUNDS WERE FOUND TO BE VERY UNSTABLE.

TABLE II

Mannich bases from 4-hydroxyquinoline

S NOS	R	MOLECULAR FORMULA	M P O ₂	FOUND			REQUIRED		
				C	H	N	C	H	N
253	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_2\text{H}_5 \\ \text{C}_2\text{H}_5 \end{matrix}$	$\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}$	324	74.0	7.8	11.5	73.8	8.2	11.5
254	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_5 \\ \text{C}_6\text{H}_5 \end{matrix}$	$\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}$	310	76.3	9.8	9.7	76.0	9.3	9.3
255	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{matrix}$	$\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}$	302	74.5	8.0	10.6	75.0	7.8	10.9
256	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_3\text{O} \\ \text{C}_6\text{H}_3\text{O} \end{matrix}$	$\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2$	320	70.0	7.2	10.8	69.8	7.0	10.8

TABLE III

Mannich bases from 5-hydroxyquinolines

S NOS	R	R ₂	MOLECULAR FORMULA	M P O ₂	FOUND			REQUIRED		
					C	H	N	C	H	N
257	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_2\text{H}_5 \\ \text{C}_2\text{H}_5 \end{matrix}$	H	$\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}$	VISCOUS OIL PICRATE 122	72.9	8.4	12.6	73.0	7.8	12.2
258	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{matrix}$	H	$\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}$	OIL PICRATE 179	74.6	8.4	11.8	74.4	7.4	11.6
259	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_3\text{O} \\ \text{C}_6\text{H}_3\text{O} \end{matrix}$	$-\text{CH}_2-\text{N} \begin{matrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{matrix}$	$\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_2$	169	66.3	7.2	12.0	66.5	7.3	12.2

TABLE IV

Mannich bases from 6 hydroxyquinoline

S NOS	R	MOLECULAR FORMULA	M P OC	FOUND			REQUIRED		
				C	H	N	C	H	N
260	$\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_2\text{H}_5 \\ \text{C}_2\text{H}_5 \end{smallmatrix}$	$\text{C}_9\text{H}_9\text{N}_2\text{O}$	VISCOUS OIL PICRATE 192	73.1	7.6	11.7	73.0	7.8	12.2
261	$\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{smallmatrix}$	$\text{C}_{13}\text{H}_9\text{N}_2\text{O}$	85°C PIC-RATE 204	74.0	7.5	11.1	74.4	7.4	11.6
262	$\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_6\text{H}_3\text{O} \\ \text{C}_6\text{H}_3\text{O} \end{smallmatrix}$	$\text{C}_{14}\text{H}_9\text{N}_2\text{O}_2$	96-98	68.8	6.7	11.3	68.8	6.6	11.5
263	$\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_8\text{H}_6\text{N} \\ \text{C}_8\text{H}_6\text{N} \end{smallmatrix}$	$\text{C}_9\text{H}_9\text{N}_2\text{O}_2 \cdot 2\text{HCl}$	HYDRO-CHLORIDE 340(4)	60.4	4.0	7.6	60.5	3.5	7.5

TABLE V

Mannich bases from 6 hydroxy 1, 2, 3, 4 tetrahydroquinoline

S NOS	R	MOLECULAR FORMULA	M P OC	FOUND			REQUIRED		
				C	H	N	C	H	N
264	$-\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_2\text{H}_5 \\ \text{C}_2\text{H}_5 \end{smallmatrix}$	$\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}$	VISCOUS LIQ	71.8	9.0	11.5	71.8	9.4	11.9
265	$-\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{smallmatrix}$	$\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}$	SOLID 172-3	73.6	9.3	11.0	73.2	8.9	11.4
266	$-\text{CH}_2-\text{N} \begin{smallmatrix} \text{C}_6\text{H}_3\text{O} \\ \text{C}_6\text{H}_3\text{O} \end{smallmatrix}$	$\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_2$	VISCOUS LIQ	67.4	8.7	11.2	67.7	8.9	11.3

on a water-bath for 3-5 hr keeping the pH at 10 by occasional addition of alkali. This was then acidified with aqueous acetic acid (10%) to pH 6 to obtain the pale brown precipitate of 5-hydroxyquinoline, mp 222°C (lit 224°C). More of this compound could be recovered from the filtrate by basification followed by heating and acidification. Total yield, 4.5 g.

Mannich bases from hydroxyquinolines The preparation of these is illustrated by the procedure for the preparation of 6-diethylaminomethyl-5-hydroxyquinoline. Minor modifications, wherever necessary, have been indicated. The final bases are listed in Tables I to VI.

TABLE VI

Mannich bases from 7-hydroxyquinoline



S. NO.	R	MOLECULAR FORMULA	M.P. °C	FOUND			REQUIRED		
				C	H	N	C	H	N
267	$\text{CH}_2 \text{N} \begin{matrix} \text{C}_2\text{H}_5 \\ \text{C}_2\text{H}_5 \end{matrix}$	$\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}$	VISCOUS LIQ. PICRATE 290-292	72	7	8	73	8	8
268	$\text{CH}_2 \text{N} \begin{matrix} \text{C}_6\text{H}_5 \\ \text{C}_6\text{H}_5 \end{matrix}$	$\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}$	VISCOUS LIQ. PICRATE 308-309	74	4	7	74	4	7
269	$\text{CH}_2 \text{N} \begin{matrix} \text{C}_6\text{H}_4 \\ \text{C}_6\text{H}_4 \end{matrix}$	$\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}$	VISCOUS LIQ. PICRATE 216-217	60	2	6	60	6	6
270	$\text{N} \begin{matrix} \text{C}_6\text{H}_5 \\ \text{C}_6\text{H}_5 \end{matrix}$	$\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}$	237-238	75	3	4	75	3	4

6-Diethylaminomethyl-5-hydroxyquinoline (S. No. 257)

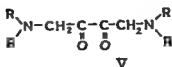
5-Hydroxyquinoline (1.5 g) was dissolved in ethanol (30 cc.) cooled at 4°C and diethylamine (1.2 cc, 1 mole) was added. A solution of formaldehyde

obtained in a pure form by removing ethanol from the reaction mixture and crystallising from a suitable solvent. Chromatography is not necessary for their purification.

Serial nos. 250-252 were characterised as hydrochlorides (nos. 260, 261, and 267) were prepared by using 3 moles each of formaldehyde and the amine per mole of the hydroxy compound.

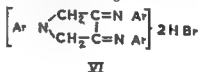
SUMMARY

Synthesis of Mannich bases of 4-, 5-, 6- and 7-hydroxyquinolines, 4-hydroxyquinoline and 1,2,3,4-tetrahydro-6-hydroxyquinoline as potential amoebicides is described.



Compounds of this type were prepared by reacting the appropriate secondary amines with 1,4-dibromo-2,3-butanedione; the latter being obtained by bromination of diacetyl (4).

Reaction of 1,4-dibromo-2,3-butanedione with aromatic primary amines proceeded in a different manner whereby the di-anils (VI) of 1-aryl-3,4-pyrroli-dinedione were obtained. Similar anils were also reported by Lehr *et al.* (5,6,7) who obtained such compounds by condensing diacetyl with various primary amines in a search for anti-tubercular drugs.



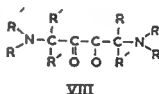
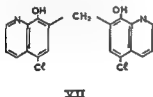
Reaction of 1,4-dibromo-2,3-butanedione with pyrrole hydrazine and ethylene diamine gave dark resinous products. Reaction with piperazine, N-methyl and N-ethyl aniline, *o*-phenylenediamine and 2-aminothiazole gave hygroscopic products which could not be analyzed satisfactorily. Reaction

4,7-phenan

V) can also
paration of

compounds of the type (VIII) where R = Me, Et or Ph is in progress and this work will be published later.

The biological screening of these compounds is in progress.

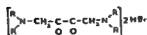


EXPERIMENTAL

All melting points are uncorrected.

absolute alcohol and ether. Most of the compounds are very hygroscopic.

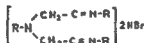
TABLE I



No	R	Mol formula	M p °C	Analysis %	
				Found	Required
1	Methyl	$\text{C}_5\text{H}_{12}\text{N}_2\text{O}_2\text{Br}_2$	Hygroscopic		
2	Ethyl	$\text{C}_{13}\text{H}_{26}\text{N}_2\text{O}_2\text{Br}_2$	Darkens at 150, melts at 172 (d)	71	72
3	n Propyl	$\text{C}_{19}\text{H}_{38}\text{N}_2\text{O}_2\text{Br}_2$	259-60 (d)	70	67
4	n Butyl	$\text{C}_{25}\text{H}_{50}\text{N}_2\text{O}_2\text{Br}_2$	Darkens at 140, melts at 318 (d)	58	58
5	iso-Butyl	$\text{C}_{20}\text{H}_{40}\text{N}_2\text{O}_2\text{Br}_2$	308 (d)	59	58
6	n Amyl	$\text{C}_{31}\text{H}_{62}\text{N}_2\text{O}_2\text{Br}_2$	Darkens at 200 and starts decomposing	46	50
7	iso Amyl	$\text{C}_{26}\text{H}_{52}\text{N}_2\text{O}_2\text{Br}_2$	Darkens at 190 melts at 312 (d)	51	50
8	Cyclo Hexyl	$\text{C}_{26}\text{H}_{48}\text{N}_2\text{O}_2\text{Br}_2$	> 320	49	46
9	Benzyl	$\text{C}_{27}\text{H}_{30}\text{N}_2\text{O}_2\text{Br}_2$	250	49	44
10	Piperidyl	$\text{C}_{24}\text{H}_{40}\text{N}_2\text{O}_2\text{Br}_2$	Starts decomposing at 139	72	68
11	Morpholyl	$\text{C}_{23}\text{H}_{36}\text{O}_3\text{N}_2\text{Br}_2$	Darkens at 170 melts at 199 (d)	66	67
12	1, 2, 3, 4 Tetrahydro quinolyl	$\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_2\text{Br}_2$	166 (d)	60	55
13	Ethyleneimino	$\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{Br}_2$	110 (d)	89	85
14	Indolyl	$\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_2\text{Br}_2$	315 (d)	61	58
15	2 Aminopiperidyl	$\text{C}_{24}\text{H}_{40}\text{N}_2\text{O}_2\text{Br}_2$	Effervesces with decomposition at 115-16°	156	150

d = decomposition

TABLE II



No	R	Mol formula	M p °C	Analysis %	
				Found	Required
1	Phenyl	$\text{C}_{21}\text{H}_{20}\text{N}_2\text{Br}_2$	225-26	85	86
2	p-Chlorophenyl	$\text{C}_{20}\text{H}_{17}\text{N}_2\text{Cl}_2\text{Br}_2$	215 (d)	68	70
3	p-Methoxyphenyl	$\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_2\text{Br}_2$	211-12 (d)	66	72
4	p-Methylphenyl	$\text{C}_{20}\text{H}_{19}\text{N}_2\text{Br}_2$	285 (d)	71	79
5	β-Phenylethyl	$\text{C}_{22}\text{H}_{22}\text{N}_2\text{Br}_2$	234 36, d	64	73
6	Benzyl	$\text{C}_{21}\text{H}_{20}\text{N}_2\text{Br}_2$	Softens at 167 melts at 188 (d)	73	78
7	p-Methoxybenzyl	$\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_2\text{Br}_2$	189-90 (d)	62	67
8	p-Tolyl benzyl	$\text{C}_{22}\text{H}_{22}\text{N}_2\text{Br}_2$	316-17 (d)	57	58
9	cyclo-Hexyl	$\text{C}_{23}\text{H}_{40}\text{N}_2\text{Br}_2$	Softens at 161 melts at 172-73 (d)	82	83
10	2, 5-Dichlorophenyl	$\text{C}_{20}\text{H}_{16}\text{Cl}_2\text{N}_2\text{Br}_2$	243 (d)	59	60
11	o-Methoxyphenyl	$\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_2\text{Br}_2$	181-82 (d)	69	72
12	o-Naphthyl	$\text{C}_{21}\text{H}_{17}\text{N}_2\text{Br}_2$	279 (d)	69	65
13	β-Naphthyl	$\text{C}_{22}\text{H}_{20}\text{N}_2\text{Br}_2$	209-10 (d)	61	65
14	o-Chlorophenyl	$\text{C}_{20}\text{H}_{17}\text{N}_2\text{Cl}_2\text{Br}_2$	197-98 (d)	65	70
15	Benzal ne	$\text{C}_{21}\text{H}_{20}\text{N}_2\text{Br}_2$	734 (d)	97	97
16	DDN	$\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{N}_2\text{Br}_2\text{S}_2$	Effervesces at 24°C (d)	74	72

SUMMARY

Several 2,3-butanediones carrying tertiary nitrogen atoms in 1 and 4 positions have been synthesized as potential amoebicidal agents. Reaction of 1,4-dibromo-2,3-butanedione with various aromatic primary amines gave the corresponding 3,4-dianils of 1-aryl-3,4-pyrrolidinedione.

ACKNOWLEDGMENT

Thanks are due to Shri P. N. Khanna for microanalyses.

REFERENCES

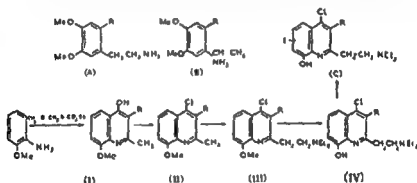
1. D. R. J. C. S. K. M. J. D. 1950. *U. S. A. 2,3* 1950.
2. " "
3. " " 1951.
4. " " 1951.
5. " " 1951.
6. " " 1951.
7. " " 1951.
8. " " 1951.

SEARCH FOR NEW AMOEBICIDES PART IV

K. L. PATHAK AND B. PATHAK

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Some halo-8-hydroxyquinolines, like chinfon, vioform and diodoquine possess pronounced amoebicidal activity. Burckhalter and Edgerton (1) observed high *in vitro* amoebicidal activity in 5-chloro-7-diethylaminomethyl-8-quinolinol. It was observed by Kaushiva (2) that high *in vitro* amoebicidal activity exists among compounds of the types A and B where R = *n* butyl or *n* hexyl. In view of these observations it has been considered worthwhile to synthesise and investigate amoebicidal activity in compounds of the type C. These compounds have been



compound C R = C₁₀H₁₃ could not be crystallized

EXPERIMENTAL

(Melting points are uncorrected. Petroleum ether used had the b.p. 60-80°).

2 = Dimethyl 4-hydroxy-3-methoxyquinoline (I, R = CH₃)

A mixture of *o*-aminidine (1 mol) 2-methyl-acetoacetic ester (1 mol) and a few drops of dilute HCl was kept overnight and then heated in an oil-bath at

120 to 130° in a Dean and Stark apparatus till no more water collected. Benzene was then distilled off and the residual viscous mass was poured into boiling diphenyl oxide. After boiling for 15 min, the mass was cooled, diluted with petroleum ether, kept overnight, filtered and washed with fresh petroleum ether.

The yield was 55%. It was crystallized from alcohol in white needles, m p 289-91° (Found C, 70.5, H, 6.6 $C_{12}H_{13}O_2N$ requires, C, 70.94, H, 6.4%).

2-Methyl 3-ethyl-4-hydroxy-8-methoxyquinoline (I, $R=C_2H_5$)

It was prepared from a mixture of *o*-anisidine and 2-ethyl acetoacetic ester in 65% yield. It was crystallized from alcohol in white needles, m p 288° (Found C, 71.5, H, 6.7 $C_{13}H_{15}O_2N$ requires C, 71.88, H, 6.91%).

2-Methyl-3-n-butyl-4-hydroxy-8-methoxyquinoline (I, $R=C_4H_9$)

It was prepared from a mixture of *o*-anisidine and 2-n-butyl-acetoacetic ester in 52% yield. It separated from alcohol in white needles, m p 196-97° (Found C, 73.1, H, 7.5 $C_{16}H_{19}O_2N$ requires C, 73.47, H, 7.75%).

2-Methyl 3-n-amyl-4-hydroxy-8-methoxyquinoline (I, $R=C_5H_{11}$)

It was prepared from *o*-anisidine and 2-n-amyl-acetoacetic ester in 55% yield, white needles (alcohol), m p 165° (Found C, 73.9, H, 7.9 $C_{18}H_{21}O_2N$ requires C, 74.13, H, 8.12%).

2-Methyl 3-n-hexyl-4-hydroxy-8-methoxyquinoline (I, $R=C_6H_{13}$)

It was prepared from *o*-anisidine and 2-n-hexyl acetoacetic ester in 42% yield. It crystallized from alcohol in white needles, m p 152° (Found C, 74.4, H, 8.1 $C_{17}H_{21}O_2N$ requires C, 74.71, H, 8.42%).

2,3-Dimethyl-4-chloro-8-methoxyquinoline (II, $R=CH_3$)

A mixture of I ($R=CH_3$, 15g), $POCl_3$ (35 cc) and PCl_5 (3 g) was heated in an oil-bath at 125-30° for 3 hr. The product was then cooled and poured on to ice. The aqueous solution was partly neutralized, purified with charcoal and basified with ammonia under cooling. The chloro compound separated out as fine needles and was crystallized from petroleum ether in colourless fine needles, m p 132-33°, yield, 14 g (Found C, 64.8, H, 5.3 $C_{11}H_{11}ONCl$ requires C, 65.01, H, 5.41%).

Its picrate crystallized from alcohol in deep yellow needles, m p 204-206°.

2-Methyl 3-ethyl-4-chloro-8-methoxyquinoline (II, $R=C_2H_5$)

Crystallized from petroleum ether in colourless fine needles, m p 115° (Found C, 66.0, H, 5.9 $C_{11}H_{11}ONCl$ requires C, 66.24, H, 5.94%). The picrate crystallized from alcohol in yellow needles, m p 163-65°.

■ Methyl 3-n-propyl-4-chloro-8-methoxyquinoline (II, $R=C_3H_7$)

Crystallized from petroleum ether in colourless needles, m p 122-23° (Found C, 67.0, H, 6.1 $C_{14}H_{16}ONCl$ requires C, 67.33, H, 6.41%). The picrate crystallized from alcohol in yellow needles, m p 165-66°.

° (Found
The Picrate

2-Methyl-3-n-amyl-4-chloro-8-methoxyquinoline (II, $R=C_5H_{11}$)

Crystallized from petroleum ether in colourless needles, m p 101-102°.

(Found C 68.7 H 7.0 $C_{16}H_{20}O$ NCl requires C, 69.18 H 7.2%) The *picrate* crystallized from alcohol in yellow needles m p 161-62°

2 Methyl 3 n hexyl 4 chloro 8 methoxyquinoline (II R- C_6H_{13})

Crystallized from petroleum ether in colourless needles m p 96-97°
(Found C 69.6 H 7.2 $C_{21}H_{29}ONCl$ requires C 69.98 H 7.55%) Its *picrate* crystallized from alcohol in yellow needles, m p 130-32°

2 β Diethylaminoethyl 3 methyl 4 chloro 8 methoxyquinoline (III R- CH_3)

A mixture of II (R- CH_3 , 6.6 g) diethylamine hydrochloride (3.3 g) paraformaldehyde (1.8 g) and 3 drops of concentrated HCl in absolute alcohol (10 cc) was heated on a water bath. After an hour a further amount (1 g) of paraformaldehyde was added and heating continued for another 2 hr. Alcohol was removed on a water bath the residue was dissolved in water basified with caustic soda and the base was extracted with benzene. The benzene solution was dried over anhydrous Na_2SO_4 and dry HCl gas was passed in it when the hydrochloride separated. It was filtered and washed with dry acetone. The substance was highly hygroscopic. The *picrate* was crystallized from large volume of alcohol in brown needles m p 195-96° (Found N 13.4 $C_{23}H_{30}O_6N_5Cl$ requires N 13.07%)

(Found
79°)

2 β Diethylaminoethyl 3 n propyl 4 chloro 8 methoxyquinoline (III R- C_3H_7)

The *picrate* crystallized from alcohol in yellow needles m p 158-60°
(Found N 12.6 $C_{25}H_{30}O_6N_5Cl$ requires N 12.43%)

(Found
151-52°)

2 β Diethylaminoethyl 3 n amyl 4 chloro 8 methoxyquinoline (III R- C_5H_{11})

The *picrate* crystallized from alcohol in yellow needles m p 129-30°
(Found N 12.1 $C_{27}H_{34}O_6N_5Cl$ requires N 11.83%)

2 β Diethylaminoethyl 3 n hexyl 4-chloro 8 methoxyquinoline (III, R- C_6H_{13})

The *picrate* crystallized from alcohol in yellow needles m p 139-40°
(Found N 11.4 $C_{29}H_{36}O_6N_5Cl$ requires N 11.55%)

2 β Diethylaminoethyl 3 methyl 4 chloro-8 hydroxyquinoline (IV, R- CH_3)

A mixture of crude II¹ and water (28 cc) was to ice partly neutralized monia under cooling. The caustic soda. On acidification compound separated out. The *picrate* crystallized from alcohol in yellow needles m p 190-92°
(Found N 12.3 $C_{25}H_{30}O_6N_5Cl$ requires N 12.43%)

(IV, R- C_2H_5)
low needles m p 190-92°
(7%)

2 β Diethylaminoethyl 3 n propyl 4-chloro 8 hydroxyquinoline (IV, R- C_3H_7)

The *picrate* crystallized from alcohol in yellow needles m p 170-71°
(Found N 12.8 $C_{27}H_{34}O_6N_5Cl$ requires N 12.74%)

2 β Diethylaminoethyl 3 n butyl 4 chloro-8 hydroxyquinoline (IV, R- C_4H_9)

The *picrate* crystallized from alcohol in yellow needles m p 115-16°
(Found N 12.3 $C_{28}H_{36}O_6N_5Cl$ requires N 12.43%)

120 to 130° in a Dean and Stark apparatus till no more water collected. Benzene was then distilled off and the residual viscous mass was poured into boiling diphenyl oxide. After boiling for 15 min, the mass was cooled, diluted with petroleum ether, kept overnight, filtered and washed with fresh petroleum ether.

The yield was 55%. It was crystallized from alcohol in white needles, m p 289-91° (Found C, 70.5, H, 6.6 $C_{12}H_{13}O_2N$ requires, C, 70.94, H, 6.4%)
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It was prepared from a mixture of *o*-anisidine and 2-ethyl acetoacetic ester in 65% yield. It was crystallized from alcohol in white needles, m p 288° (Found C, 71.5, H, 6.7 $C_{13}H_{15}O_2N$ requires C, 71.88, H, 6.91%)

2-Methyl-3-*n*-butyl-4-hydroxy-8-methoxyquinoline (I, $R=C_4H_9$)

It was prepared from a mixture of *o*-anisidine and 2-*n*-butyl-acetoacetic ester in 52% yield. It separated from alcohol in white needles, m p 196-97° (Found C, 73.1, H, 7.5 $C_{15}H_{19}O_2N$ requires C, 73.47, H, 7.75%)

2-Methyl-3-*n*-amyl-4-hydroxy-8-methoxyquinoline (I, $R=C_5H_{11}$)

It was prepared from *o*-anisidine and 2-*n*-amyl-acetoacetic ester in 55% yield, white needles (alcohol), m p 165° (Found C, 73.9, H, 7.9 $C_{16}H_{21}O_2N$ requires C, 74.13, H, 8.12%)

2-Methyl-3-*n*-hexyl-4-hydroxy-8-methoxyquinoline (I, $R=C_6H_{13}$)

It was prepared from *o*-anisidine and 2-*n*-hexyl acetoacetic ester in 42% yield. It crystallized from alcohol in white needles, m p 152° (Found C, 74.4, H, 8.1 $C_{17}H_{23}O_2N$ requires C, 74.71, H, 8.42%)

2,3-Dimethyl-4-chloro-8-methoxyquinoline (II, $R=CH_3$)

A mixture of I ($R=CH_3$, 15g), $POCl_3$ (35 cc) and PCl_5 (3 g) was heated in an oil-bath at 125-30° for 3 hr. The product was then cooled and poured on to ice. The aqueous solution was partly neutralized, purified with charcoal and basified with ammonia under cooling. The chloro compound separated out as fine needles and was crystallized from petroleum ether in colourless fine needles, m p 132-33°, yield, 14 g (Found C, 64.8, H, 5.3 $C_{12}H_{13}ONCl$ requires C, 65.01, H, 5.41%)

Its picrate crystallized from alcohol in deep yellow needles, m p 204-206°

2-Methyl-3-ethyl-4-chloro-8-methoxyquinoline (II, $R=C_2H_5$)

Crystallized from petroleum ether in colourless fine needles, m p 115° (Found C, 66.0, H, 5.9 $C_{13}H_{14}ONCl$ requires C, 66.24, H, 5.94%) The picrate crystallized from alcohol in yellow needles, m p 163-65°

2-Methyl-3-*n*-propyl-4-chloro-8-methoxyquinoline (II, $R=C_3H_7$)

Crystallized from petroleum ether in colourless needles, m p 122-23° (Found C, 67.33, H, 6.41%) The picrate crystallized from alcohol in yellow needles, m p 165-66°

1°. (Found
The Picrate

2-Methyl-3-*n*-amyl-4-chloro-8-methoxyquinoline (II, $R=C_5H_{11}$)

Crystallized from petroleum ether in colourless needles, m p 101-102°.

(Found C, 68.7, H, 7.0 $C_{26}H_{20}O$ NCl requires C, 69.18, H 7.2%) The picrate crystallized from alcohol in yellow needles m p 161-62°

picrate crystallized from alcohol in yellow needles, m p 150-52°

paraformaldehyde was added and heating continued for another 2 hr. Alcohol was removed on a water bath the residue was dissolved in water basified with caustic soda and the base was extracted with benzene. The benzene solution was dried over anhydrous calcium chloride separated and the residue was highly hygroscopic in brown needles N, 13.07%)

2 β Diethylaminoethyl 3 ethyl 4 chloro 8 methoxyquinoline (III, R C_2H_5)

The picrate crystallized from alcohol in brown needles m p 178-79° (Found N, 13.1, $C_{26}H_{28}O_6N_2Cl$ requires N, 12.74%)

2 β Diethylaminoethyl 3 n propyl 4 chloro 8 methoxyquinoline (III R- C_3H_7)

The picrate crystallized from alcohol in yellow needles, m p 158-60° (Found N, 12.6 $C_{28}H_{30}O_6N_2Cl$ requires N, 12.43%)

(R- C_4H_9)

needles m p 151-52°

2 β Diethylaminoethyl 3 n amyl 4 chloro 8 methoxyquinoline (III R- C_5H_{11})

The picrate crystallized from alcohol in yellow needles, m p 129-30° (Found N 12.1 $C_{30}H_{34}O_6N_2Cl$ requires N 11.83%)

II, R C_6H_{13})

needles m p 139-40°

2 β Diethylaminoethyl 3 methyl 4 chloro 8 hydroxyquinoline (IV, R- CH_3)

A mixture of crude product and water (28 cc) was added to ice, partly neutralized with caustic soda under cooling. The compound separated out. The picrate crystallized from alcohol in yellow needles, m p 190-92° (Found N, 13.6 $C_{22}H_{24}O_6N_2Cl$ requires N, 13.07%)

2 β Diethylaminoethyl 3 ethyl-4 chloro 8-hydroxyquinoline (IV, R- C_2H_5)

The picrate crystallized from alcohol in yellow needles, m p 190-92° (Found N 13.1 $C_{24}H_{26}O_6N_2Cl$ requires N, 13.07%)

(IV, R- C_3H_7)

needles, m p 170-71°

%)

2 β Diethylaminoethyl 3 n butyl 4 chloro-8 hydroxy quinoline (IV, R- C_4H_9)

The picrate crystallized from alcohol in yellow needles, m p 115-16° (Found N, 12.3 $C_{26}H_{28}O_6N_2Cl$ requires N, 12.43%)

2- β -Diethylaminoethyl-3-*n*-amyl-4-chloro-8-hydroxyquinoline (IV, R = C₅H₁₁):

The picrate crystallized from alcohol in yellow needles, m p 125-26°. (Found N, 12.5. C₂₈H₃₂O₂N₂Cl requires, N, 12.12%).

2- β -Diethylaminoethyl-3-*n*-hexyl-4-chloro-8-hydroxyquinoline (IV, R = C₆H₁₃):

The picrate crystallized from alcohol in yellow needles, m p. 126-27°. (Found N, 12.1. C₂₉H₃₄O₂N₂Cl requires N, 11.82%).

a period of half an hour. The mass was left overnight, mixed, triturated with a solution of dilute sodium thiosulphate and ammonia. The pasty greenish brown mass was then dissolved in benzene, the benzene solution was dried (Na₂SO₄). The monohydrochloride was prepared by passing dry HCl gas into the benzene solution under cooling. It crystallized from a mixture of absolute alcohol and ethyl acetate, m.p. 185-86°. (Found I, 27.1. C₁₈H₂₁ON₂Cl₂I requires I, 27.9%)

2- β -Diethylaminoethyl-3-ethyl-4-chloro-5 (or 7)-iodo-8-hydroxyquinoline (C, R = C₂H₅):

The hydrochloride crystallized from a mixture of absolute alcohol and ethyl acetate, m p. 184-86°. (Found I, 26.6. C₁₇H₂₃ON₂Cl₂I requires I, 27.08%). The hydroiodide crystallized from alcohol, m p. 166-68°.

2- β -Diethylaminoethyl-3-*n*-propyl-4-chloro-5 (or 7)-iodo-8-hydroxyquinoline (C, R = C₃H₇):

The hydroiodide crystallized from absolute alcohol, m p 177-79°. (Found: I, 43.8. C₁₈H₂₃ON₂ClI₂ requires I, 44.2%)

2- β -Diethylaminoethyl-3-*n*-butyl-4-chloro-5 (or 7)-iodo-8-hydroxyquinoline (C, R = C₄H₉):

The hydroiodide crystallized from absolute alcohol, m p 172-73°. (Found: I, 43.05. C₁₉H₂₅ON₂ClI₂ requires I, 43.1%)

SUMMARY

Synthesis of 2- β -diethylaminoethyl 3-alkyl-4-chloro-5 (or 7)-iodo-8-hydroxy-quinolines having alkyl substitution of methyl, ethyl, *n*-propyl, *n*-butyl and *n*-amyl groups have been effected with a view to observing their amoebicidal activity.

ACKNOWLEDGMENT

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REFERENCES

1. Burckhalter, J. H., and Edgerton, W. H. (1951), *J. Amer. Chem. Soc.*, **73**: 4837.
2. Kaushiva, B. S. (1957), *J. Sci. Industr. Res.*, **16C**: 224.

COMPARATIVE MICROBIOLOGICAL STUDIES OF AMOEBICIDES
WITH SPECIAL REFERENCE TO THE DERIVATIVES OF
PHENANTHROLINE-QUINONE

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The amoebicides in common clinical use to day have been classified by Armengaud and Blanc (1) according to the scheme given in Table I

TABLE I

Classification of the amoebicides

- 1 "Tissue amoebicides"
Emetine
Cinchonine
Chloroquine
Preparations with very high iodine content (X ray contrast media)
- 2 "Contact amoebicides"
Phenanthroline-quinone (PQ)
Iodoxyquinoline
Emetine-bismuth iodide
Arsenical preparations etc
- 3 "Medicaments directed against factors promoting amoebic disease"
Antibiotics

1 To the first group belong those drugs which are active against amoebae situated within the tissues of the host

2 The second group comprises those compounds which for the most part are poorly soluble and which are active within the intestinal lumen and superficially in the mucosa and in lesions thereof

The third group of substances owe their activity to their ability to influence the intestinal milieu in a more or less specific way, especially through their effect on the abnormal flora associated with and favourable to amoebic infection

This attempt at classification is useful in so far as it emphasises various factors which contribute to the practical efficacy of a given drug. It is mainly based on clinical experience with well known preparations but also depends on a knowledge of their properties gained from experimental work. Therefore, in order to place new, highly active substances into the appropriate place in such a scheme, a consideration of their characteristics and mode of action as indicated by comparative experimental studies with other amoebicides is of value, as also are early clinical results

The following are some relevant findings with two members of a new synthetic group of amoebicides—the phenanthroline-quinones (2, 6, 9) taken in comparison with data relating to other well known remedies

The antiparasitic and antibacterial activity of phenanthroline quinone (PQ) and its semicarbazone in comparison with that of other amoebicides

Many amoebicides are not only active against *E. histolytica*, but are also clinically useful for the treatment of intestinal disorders due to the presence of flagellates (*Lambia intestinalis*) or ciliates (*Balantidium coli*)

Emetine and also chloroquine whose activity against amoebae is well known, may in addition be used for the treatment of trematode infection of the liver (*Fasciola hepatica*, *Clonorchis sinensis*) (3)

As will be seen from the Table II the new substance PQ is not only active against flagellates and ciliates (i.e., *Lambia*, *Balantidium*, *Chilomastix* and also *Trichomonas*) (4-5) but like emetine and chloroquine it has also been shown by Coudert and Garin (6) to possess clinical activity against *Fasciola hepatica*

TABLE II

The clinical spectrum of anti parasitic activity of a number of amoebicides

Amoebicide	Flagellates	Established activity against		Nematodes
		Ciliates	Trematodes	
Phenanthroline quinone	<i>Lambiasis</i> <i>Trichomonas intestinalis</i>	Balantidiasis	Fascioliasis	Mouse <i>Syphacia obvelata</i>
Chlortetracycline	<i>Trichomonas vaginalis</i>	Balantidiasis		
Chloroquine			Clonorchiasis	
Emetine			Fascioliasis	Trichuriasis
Carbarsone	<i>Trichomonas vaginalis</i>	Balantidiasis		

When we come to nematodes, emetine is active against *Trichuriasis* (7), whereas PQ has been shown experimentally to be active against the mouse pin worm *Syphacia obvelata*. Other animal parasites against which PQ is also active, are *Trichomonas muris* and *T. criceti*

Thus PQ displays a broader spectrum of anti parasitic action than other

amoebicides a fact which, although of general interest, does not add significantly to our knowledge of its special activity as an amoebicide

(1) *Amoebicidal action in vitro*

Amoebicidal activity may be assessed in various types of experiment

Amongst others we have used a method by which it is possible to observe the appearance of amoebae microscopically during continued exposure to known concentrations of active substance (9) Living amoebae, together with the

apparently undamaged immobile forms are observable

The amoebicidal potency of various substances can be expressed in terms of the threshold concentration necessary to kill the amoebae In this connection the choice of medium plays an important part

At this stage it is interesting to digress a little to discuss a matter of theoretical interest concerning the role of certain heavy metal ions in the culture medium which may have an important bearing on the mechanism of action Certain constituents such as metal ions may affect the activity of amoebicides in different

zone is increased by the presence of Cu^{++} but reduced by Fe^{++} whereas chlortetracycline for instance is only inhibited by Fe^{++} and not affected by Cu^{++} In the case of the other compounds listed the presence or absence of ions of these two metals does not affect their activity

TABLE III

Influence of Cu and Fe sulphate on the in vitro activity of various amoebicides

Amoebicide	Effect of metal ions	
	Cu^{++}	Fe^{++}
PQ Sem carbazole	Synergism	Antagonism
Iodochloroxiquinolone	~	~
Chlortetracycline	~	Antagonism
Carbazone	~	~
Fumagillin	~	~
Emetine	~	~

It should be pointed out that this effect of metallic ions on amoebicidal

in vitro the following results were obtained from experiments using a standard medium with 10% added serum, but without the addition of metallic salts

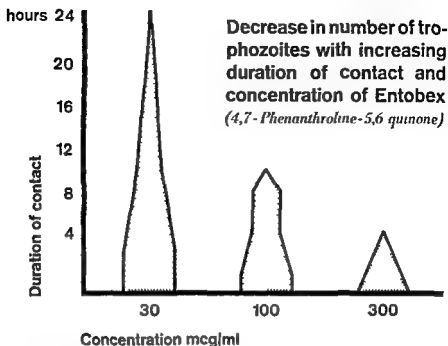


Fig 1 Diminution in the number of mobile amoebae as the duration of contact with various concentrations of amoebicide (PQ) increases (Breadth of columns proportional to percentage of survivors)

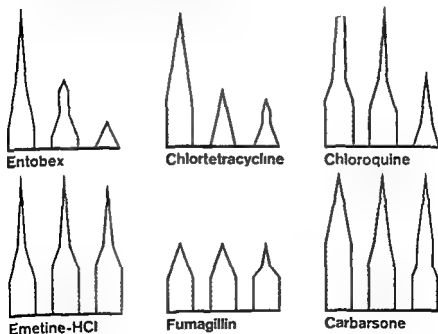


Fig 2 Comparison between amoebicides by reference to their intensity of effect at identical concentrations

Since in the lethal action of an amoebicide the time factor is of importance, it was considered valuable to record the progressive decline in the number of motile forms of amoebae as the duration of exposure to the amoebicide increased (Fig 1)

The comparative potencies of the various amoebicides as indicated by this type of experiment are best represented graphically in Fig 2. Three of the 6 amoebicides namely PQ, chlortetracycline and chloroquine differ significantly from the others (emetine, fumagillin and carbarsone)

In particular, PQ and chlortetracycline are very similar, the concentration which inactivates all amoebae in 24 hr is in both cases 30 mcg/cc and a three-fold increase in concentration leads to an almost identical reduction in the contact time necessary to inactivate all organisms. Chloroquine is about 3 times less active than these. In the case of emetine the concentration activity relationship is different, and this is also the case for carbarsone both of which substances within the range examined failed to kill amoebae more rapidly as the concentration was increased. Fumagillin belongs to the same type, but is more rapid in action than emetine and also the above mentioned preparations at least in the lowest of concentrations tested. The concentration activity relationship however, was the same as that for emetine within the range tested.

By this method the activity of various substances is more exactly defined than by the simple determination of the minimum effective concentration. However, in spite of the qualitative differences in the mode of action of various preparations thus indicated there were no corresponding differences detectable in the morphological characteristics during the period leading to the death of the organisms as seen under the microscope. This raises the question of whether some of the differences observed might not be explained by an indirect effect through an influence on the accompanying flora.

It was therefore of interest to consider this in more detail.

(2) Antibacterial action in vitro

Lamy and Molinari (10) have shown that the antibiogramme* of the concomitant flora of various strains of amoebae varies. That is to say, the spectrum of sensitivity for a given mixture of concomitant flora towards a number of antibiotics is typical for the specific mixture of organisms associated with a particular strain of amoeba.

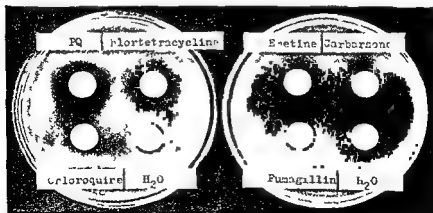


Fig. 3 Comparison between amoebicides by reference to their antibacterial activity (zones of growth inhibition in plate tests)

It was, therefore, pertinent to know the antibacterial action of the chosen amoebicides against the concomitant bacteria of our laboratory strain of *E. histolytica*. We have submitted the six above-mentioned amoebicides to the disc test against the mixed flora concerned, and obtained the results depicted in (Fig. 4).

1% concentration

The antibacterial activity of the first three and the inactivity of the second three is in accordance with the division which we have previously described on the basis of the time-concentration relationship in the direct anti-parasitic activity and in from th

Th does not adequately characterize certain important chemotherapeutic agents. Thus the pattern of activity against a number of important bacterial species plays a prominent part. For example, PQ and chlortetracycline illustrate well this point. The latter (Fig. 4) shows an ill defined but uniform zone of inhibition

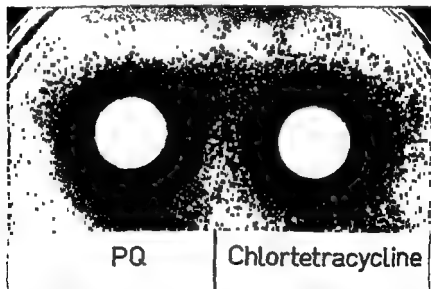


Fig. 4. Differentiation between the antibacterial activity of phenanthroline-quinone (PQ) and that of chlortetracycline as evidenced by the zones showing growth inhibition of a mixed flora.

experiments

As supplement to Table II giving the spectrum of anti-parasitic action, it may be seen from Table IV that PQ, iodochloroxyquinoline and chlortetracy-

TABLE IV
Antibacterial spectrum of some amoebicides

Preparation	Threshold bacteriostatic concentration ($\mu\text{g/cc}$) against					
	<i>Staph aureus</i>	<i>Strept haemolyt</i>	<i>Strept farcalis</i>	<i>Esch coli</i>	<i>Salmon typhi</i>	<i>Shig sonnei</i>
Phenanthroline-quinone	5	5	10	25	10	10
Iodo-chloroxyquinoline	5	5	50	25	50	5
Chlortetracycline	1	0.5	1	5	5	5
Chloroquine	>1000	>1000	>1000	>1000	1000	1000
Emetine	>1000	>1000	>1000	1000	1000	1000
Fumagillin	>100	>100	>100	>100	>100	>100

cline possess an extensive anti bacterial spectrum against various gram positive and gram-negative bacteria (6). As judged by the minimum bacteriostatic concentration chlortetracycline is the most active of this group of compounds. In contrast, the so called specific amoebicides emetine, fumagillin and chloroquine are practically inactive also against these bacteria.

The question of whether the mode of action of various amoebicides depends essentially on the presence or absence of an antibacterial component, is not only

will also differ in their mode of action *in vivo*.

However, since in animal experiments other properties such as absorption, excretion, tissue concentration etc., are also of importance, the analysis of mechanisms of chemotherapeutic activity is made more difficult.

In attempting a comparison *in vivo* of the value of two or more amoebicides the ratio of their minimum therapeutically effective doses is relevant.

TABLE V
The minimum effective dose of some amoebicides in experiments on rats

Amoebicide	Effective dose mg/kg*	Amoebicidal effect (%)	Toxicity mg/kg** (mouse)	Effective dose
				Toxic dose
Phenanthroline	20	78	300	0.07-0.17
quinone	50	100		
Chlortetracycline	50	86	>2000	<0.05
	100	100		
Chloroquine	100	50-100	250	>0.4
Emetine	5	100	10	0.5
Fumagillin	1	70	500	0.002-0.004
	2	100		
Carbarsone	250	70	500	>0.5

* Single daily dose p.o. on 4 consecutive days. Assessment on 5th day.

** Single daily dose p.o. on 5 consecutive days. Duration of observation: 10 days.

(3) Amoebicidal activity in the rat intestine

The data reproduced in Table V, which were worked out in our laboratories using the method of Jones (1947) are designed to provide a uniform basis for

comparison. In this respect, it is of interest that the values obtained for the

takes into account the margin between the minimal therapeutic dose and the toxic dose in the mouse, as shown in Table V, the order of precedence alters somewhat, in as much as emetine becomes one of the last in the list and PQ comes established acute toxicity,

to be considered as regards the *in vivo* activity of the other amoebicides. In

(4) Effect on the intestinal flora of rats

Although the task of making a quantitative and qualitative analysis of the

TABLE VI

Effect of amoebicidal doses of PQ, chlortetracycline and emetine on the faecal flora of rats

Amoebicide	Dose* mg/kg	Change in the number of bacteria in the faeces		
		Coliform bacteria	Other aerobic bacteria	Fungi
PQ	50	Increase	Increase	No change
Chlortetracycline	100	(Decrease)	(Decrease)	Increase
Emetine	5	No change	No change	No change

* Daily oral dose given 5 times within 5 days (Where the result is quoted in brackets the probability of error is $P < 0.05$ Wilcoxon's test)

at all

Data from our laboratories (9) indicate that the intestinal flora of rats reacts to doses of PQ in various phases. An initial reduction in the number of bacteria is followed by an increase to levels higher than those determined prior to treatment. Although similar alterations in the number of bacteria have also been encountered after treatment with other antibacterial substances it should be noted that PQ has a particularly pronounced effect in this respect and therefore constitutes an exception among amoebicides. The practical significance of this peculiarity, however, can only be determined in the light of further clinical and laboratory investigation. Since it is technically difficult to establish with any certainty, effects exerted upon non specific pathological intestinal flora in man, additional experimental research along these lines would seem to be indicated at the same time as studies on man (12). The numerous findings which have emerged from our experiments with the six amoebicides compared here provide renewed evidence for the assumption that the amoebicides differ in various respects as regards their mode of action. They also show that the new preparation (PQ) is one which, compared with the ordinary run of amoebicides, merits a position in a special category.

REFERENCES

1. Armenegaud M. (1959) *Lecture given at the Swiss Tropical Institute, Basle on 13.2.59*
2. Schmidt P. and Drury J. (1957) *Helv. Chim. Acta* 40: 2350
3. Watter A. M. and Heilmeyer, L. (1954) *Quoted relevant literature in Antibiotika-Tabelle* 1954
4. Sano M. 1948 *Rev. J. T. A.* 18: 649

Trop. Med. 3: 327

39: 639

INCIDENCE AND EPIDEMIOLOGY OF INTESTINAL PARASITES WITH SPECIAL REFERENCE TO INTESTINAL AMOEBIASIS AND GIARDIASIS

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It is a common experience of clinicians in the tropics and elsewhere that symptoms of vague abdominal pain and discomfort are often wrongly attributed to chronic surgical conditions of the abdominal organs, and lead to an uncalled for surgical interference. Less frequently these symptoms and signs very closely mimic those of chronic appendicitis, cholecystitis or peptic ulcer, but in fact are due to infestation with one of the intestinal parasites and when the infesta-

was prompted by such an observation.

The population of a large city was studied under the following groups:

Group A. It comprised carefully selected, apparently healthy individuals, without any abdominal symptoms, and was investigated to find out the 'parasitic spectrum' of the so-called healthy carriers.

Group B. This included persons with chronic abdominal ailments.

Group C. This consisted of cases of acute dysentery. These cases were carefully selected with the co-operation of the clinicians who referred them to us as early as possible in the course of the disease and before instituting any treatment. In addition to the normal routine stool examination these samples were cultured on desoxycholate citrate agar with a view to isolating pathogenic bacteria that might be responsible for the dysentery.

MATERIALS AND METHODS

Materials. The results of this survey are based on the results of a single

of parasites.

In collecting a fresh sample of faeces the following precautions were observed:

(i) It was made certain that the person was not taking any preparation

amination and,
were given ½ oz
prior to their
discarded. As

a rule, second or third sample of faeces, after the purgative had started to act, gave us the desired material.

(iii) The stool was collected in a sterile, clean, dry glass Petri-dish free from antiseptics, soap or other detergents. The person under investigation was instructed not to pass urine in the Petri dish as it has a lethal effect on the trophozoite forms of the protozoa (1).

Method of preparing the slide for examination As recommended by Craig (2) a small portion of faeces was thoroughly mixed with a drop of normal saline, placed on a clean slide, carefully emulsified and a cover slip placed on it. Care was taken not to make too thick emulsions, and as a guide, it was made sure that newsprints could easily be seen through it. This procedure was also repeated with a drop of Lugol's iodine. If blood or mucus was present in the faeces, small portions of these were examined as well.

The sample of faeces was concentrated by the zinc sulphate centrifugal flotation method of Faust *et al.* (3) and the float examined with a drop of Lugol's iodine.

With properly adjusted sub stage and iris diaphragm (to secure maximum definition of the trophozoite forms of the parasites) these preparations were examined by 10X ocular and 16 mm and 4 mm objectives.

Criteria for the identification of the trophozoite forms of E. histolytica For the correct diagnosis we are taught to ask two questions. Does it move? Does it contain red blood cells as inclusions? This is certainly true in cases of acute

E. histolytica very often does not contain red blood cells. We satisfied ourselves on the following points, before any structure that was changing its shape was labelled as *E. histolytica*.

- (i) Its sharper outline in contrast to coarser appearance of other amoebae.
- (ii) Well differentiated, clear, glass like ectoplasm, constituting more than one third of the whole organism, in contrast to the dull, opaque, small and indistinct zone of other amoebae.
- (iii) Its characteristic progressive motility achieved by the formation of a long finger like pseudopodia into which the granular endoplasm flows in a slow but deliberate manner. According to Craig (2) even in the absence of red blood cells as inclusions one is justified in making a diagnosis of *E. histolytica*, if this characteristic formation of pseudopodia and the flow of endoplasm is present.

In acute dysenteric cases, in addition to these, the following features were frequently seen.

- (i) Presence of red blood cells as inclusions.
- (ii) Marked directional motility, amoeba traversing long distances under the microscope.
- (iii) It assumes a slug like form and advances with a definite polarity—the advancing end being rounded and permanent, while the trailing end is more pointed and often a mass of granular debris is adherent to it.

In view of the findings of Meleney and Zuckerman (6) that small races became large on culture media, the size of the protozoa has no diagnostic value.

RESULTS

In this paper stress is laid on the incidence of the parasites and their association with signs and symptoms.

The number of cases investigated under the groups A, B and C were from the following sources.

Sources	Group A	Group B	Group C
1. Hospital cases	44	690	100
2. Members of nursing staff	57	44	
3. Factory hands	183	96	4
4. Inmates of a reformatory school	100	180	
Total	384	1,010	104

The actual incidence of the various intestinal parasites in these groups is shown in Tables I and II, and their percentages in Figs 1 and 4. Fig. 1 shows that *E. histolytica* and *G. lamblia* occur much more frequently in the faeces of group B than in those of group A. The χ^2 test shows that these differences are significant. The value of *P* (probability occurrence by chance alone) being less than 0.001 in both the cases. The incidence of symptoms and signs where these two parasites occurred either as a single or a combined infection, has been worked out in detail and is presented in Figs 2 and 3. Table II also shows the physical findings and therapeutic response in group C.

TABLE I
Showing incidence of intestinal parasites

No	Parasite	Form	Group A (384)		Group B (1,010)	
			No	Percent	No	Percent
1	<i>E. histolytica</i>	Trophozoite	38		237	
		Cyst	26	= 77	62	= 359
		Both	13	20.1	60	35.6
2	<i>Entamoeba coli</i>	Trophozoite	111		232	
		Cyst	19	= 189	59	= 425
		Both	59	49.2	131	42.1
3	<i>Endolimax nana</i>	Trophozoite		36		100
4	<i>Iodamoeba butschlii</i>	Cyst		3		11
5	<i>D. amoeba fragilis</i>	Trophozoite		10.9		10.2
				1		4
6	<i>Giardia lamblia</i>	Trophozoite	25		134	
		Cyst	17	= 44	55	= 217
		Both	2	11.5	20	21.5
7	<i>Trichomonas hominis</i>	Trophozoite		32		95
8	<i>C. lamastix mesnili</i>	Trophozoite				
		Cyst	4	= 6	8	= 20
		Both	2	9.9	12	2.0
9	Hookworm	Ova	47	12.2	168	16.6
10	<i>A. lumbricoides</i>	Ova	85	22.4	294	29.2
11	<i>Trichuris trichiura</i>	Ova	62	16.2	172	17.0
12	<i>Strongyloides stercoralis</i>	Larva	3	0.8	6	0.6
13	<i>Enterobius vermicularis</i>	Ova	10	2.6	25	2.0
14	<i>Hymenolepis nana</i>	Ova	10	2.6	25	2.5
15	<i>T. (saginata) solium</i>	Ova	1	0.3	1	0.9
16	<i>Blas-tocystis hominis</i>		65	16.9	154	15.3
17	Yeast		40	10.4	66	6.5
18	Sarcinae		96	25.0	178	17.6

TABLE II

Intestinal parasites in human population Group C—104 cases of acute dysentery

Pathogen	Initial no	Per cent	Pathogen demonstrated	Presumptive diagnosis		Pathogenic bacteria			Other parasites found					Other characters of the stool										Response to treatment		
				Flexner	C I	C II	<i>Flexus morganii</i>	<i>E. typhi</i>	<i>B. dysenteriae</i>	<i>E. coli</i>	<i>C. lamblia</i>	<i>C. merialis</i>	<i>T. hominis</i>	Hookworm	Strongyloid	Acanth	Trichina	Charcot Leyden crystals	R B C clumped	R B C movement	Acid	Alkaline	Assoc of alcoholism with liver findings		History of previous dysentery	
1 Amoebic infection only	45	44.2	46																					20	46	All responded to emetine hydrochloride, $\frac{1}{2}$ gr sub cut once a day for 7 days. One case (a medical student) relapsed repeatedly and ended up with ulcerative colitis which responded only to small repeated blood transfusions. At the end of treatment seven were still passing cysts though asymptomatic. Out of 17 people with liver findings 12 showed improvement.
2 Amoebic and bacillary mixed infection	5	48	3	2	1	1	1	1	1															5	4	All except one (typhoid carrier) needed combined therapy of emetine as in (1) and sulphaguanidine as in (3) (a and b). The typhoid carrier with coincident amoebic dysentery responded to emetine alone.
3 Bacillary dysentery	39	37.5 (a)	12	6	3	4																		12	6	(a) & (b) — Responded strikingly (48—72 hr) to sulphaguanidine.

TABLE II (Contd.)

Pathogen	Total no	Percent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria		Other parasites found		Other characters of the stool										Response to treatment						
					Flex	Coli	Proteus morgan	L typhi	B dysenteriae	E coli	G lamblia	Ck mearnsi	T hominis	Hookworm	Strongyloid	Ascari	Trichuris	Charcot Leyden crystals		RBC clumped	RBC movement	Acid	Alkaline	Assoc of alcoholism with liver findings	History of previous dysentery
4 <i>Giardia lamblia</i>	8	77.0											0	2			1	1	1	2	2	6	4	In all these colic and tenesmus was less marked, abdominal pain was more vague, and the stools contained less blood but more mucus. 0.1 megacaine t.d.s daily by mouth for five days was given. Six improved and at the end of treatment had no dysenteric symptoms nor the parasite in the stools. Two showed slight improvement and the infection persisted. In the cured series, in one leucorrhoea persisted but in another the associated iridocarcia vanished.	
5 Hookworm infec-	2	100												2									2	1	Ifenyl resorcinol, 1 gr. in capsule, on empty stomach followed by a saline purge 2 ft. 12 afterwards. Dysenteric symptoms disappeared in both the cases though pulmonary symptoms persisted. Ova detected in the stool of one of them even after treatment.

TABLE II (Contd.)

Pathogen	Total no	Per cent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria							Other parasites found		Other characters of the stool						Response to treatment					
					Flex ner	G I	G II	Proteus morganii	E typhi	B pyocyaneus	E coli	C lambdella	Cm marseillensis	T hominis	Hookworm	Strongyloid	Ascari	Trichuris	Charcot Leyden crystals		R B C clumped	R B C movement	Acid	Alkaline	Assoc of alcohol sm with liver findings
6 Strongyloides stercoralis	1	0.9	1																						Not treated
7 Ascaris lumbricoides	3	2.9	3																						Two cases were treated. The third refused treatment. Hexyl resorcinol 1 gr on empty stomach followed by a saline purge an hour afterwards. One responded well, passed several adult worms and was asymptomatic by third day, second was partially relieved. Stool of both continued to show ova. Pulmonary symptoms not relieved.

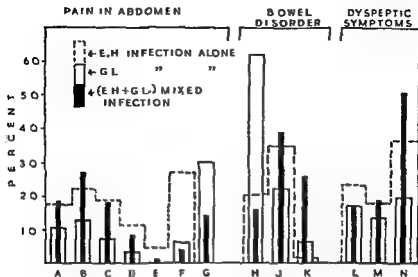


Fig 3

A Pain in epigastrium B Pain in Rt iliac fossa C Pain in Lt iliac fossa D Pain in Rt hypochondrium E Pain in Lt hypochondrium F Pain in umbilical region G Vague pain all over H Diarrhoea J Constipation K Alternate constipation & diarrhoea L Anorexia M Nausea & vomiting N Flatulence

DISCUSSION

Most of the surveys of intestinal parasites done in recent years in different localities have been of a temporary floating population, e.g., troops in various theatres of war or temporary army hospitals etc. This survey is of significance as it deals with more or less static population of a city.

Group A The incidence of various parasites in this group shows that highly

discussion. A few important parasites, however, have been picked out by several workers for such a study. *F. histolytica* has been more frequently investigated than any other.

such a group is shown in Table 14.

Group B Fig 1 reveals a few interesting features in the infection rate of groups A and B. In people with abdominal symptoms there is an appreciably higher rate of infestation with *F. histolytica*, *G. lamblia* and hookworm than in those who are symptom-free, the differences are 15.5, 10.0 and 4.4%, respectively.

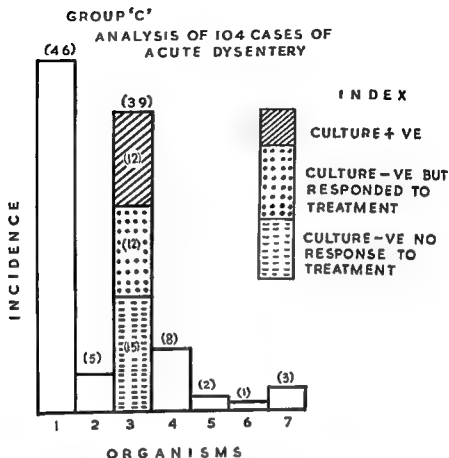


Fig 4

1 *E. histolytica* 2 *E. histolytica* + Bacillary 3 Bacillary infection 4 *Giardia lamblia*
5 Hookworm 6 *Strongyloid stercoralis* 7 *Ascaris lumbricoides*

E. coli and round-worm infection is lower in group B by 7.6 and 2.2%, respectively. Other protozoa and helminths are more or less at the same level and non-pathogens, such as *Blastocystis hominis*, yeast and sacciniae are less common in group B.

The incidence of *E. histolytica* infection in people with vague abdominal

symptoms 50 to 55% of the incidence in group B is nearly double to that in group A, and in our opinion it is an important parasite in the production of chronic and vague abdominal symptoms. In Asmara, Sofia and Giaravino (15) found only 1% people with abdominal symptoms to be infested with this parasite. In contrast to the high incidence of tapeworms (especially the *Tenia* group) infestation in the human population of other countries, their incidence in this

TABLE III

No	Investigator (Ref)	Local ty	No of cases examined	No positive	Percentage
1	Mac Adam (7)	India	351	46	13.0
2	Hardy and Spector (8)	Chicago	161	25	15.5
3	Cannan (9)	Jerusalem	180	40	20.2
4	Patel (10)	Bombay	125	54	43.3
5	Leitman and Vilinskaya (11)	Tashkent (USSR)	1 002	128	12.7
6	Shrivastav (12)	Bombay	384	77	20.2

TABLE IV

No	Investigator (Ref)	No of healthy adults investigated	Percentage
1	Kofoid quoted by Craig and Faust (14)	2 300	5.7
2	Jepps quoted by Craig and Faust (14)	971	13.2
3	Leitman and Vilinskaya (11)	1 002	17.9
4	Shrivastav (12)	384	11.5

TABLE V

No	Investigator (Ref)	Locality	Total no examined	No found positive	Percentage
1	Hardy and Spector (8)	Chicago	33	14	42.0
2	Cannan (9)	Jerusalem	400	122	30.5
3	Sofa and Chiravino (15)	Amara	700	209	29.8
4	Liebermann (16)	Natal	103	31	29.5
5	Brown (17)	N Africa & European theatres	290	63	21.8
6	Patel (10)	Bombay	101	58	58.0
7	Shrivastav (12)	Bombay	1 010	359	35.6

survey, in any group, is remarkably low but this can be easily explained. The Hindus and Muslims, who constitute the majority of the population in the city, on religious grounds do not eat either beef or pork respectively. The few cases of *Tenia* infection detected were mostly in the Christian population of the city. Among the tapeworms detected *H. nana* infestation was the commonest.

Association of parasites with signs and symptoms in group B *E. histolytica* and *G. lamblia* had the highest incidence among the pathogenic parasites in this group. Their association with the signs and symptoms as shown in Figs 2 and 3, may briefly be summarized in order of their frequency (Table VI).

It is clear from this summary that the symptom complex and signs in these two infections are remarkably different in order of frequency and in details when

TABLE VI

Parasite	Symptoms and signs in order of frequency	Details of symptoms and signs in order of frequency
<i>E. histolytica</i> alone	1 Pain (73%) 2 Dyspepsia (56.9%) 3 Disorder of bowel (55%) 4 Constitutional symptoms (36.1%)	Right iliac fossa and round about umbilicus Flatulence and anorexia Constipation Loss of weight
Signs	1 Involvement of caecum (47.3%) 2 Involvement of liver (17.1%) Common surgical condition misdiagnosed	Tender and palpable Chronic appendicitis (5.4%) Chronic peptic ulcer (5.0%)
<i>G. lamblia</i> alone	1 Bowel disorder (89.8%) 2 Pain (63.5%) 3 Dyspepsia (43.2%) 4 Constitutional symptoms (24.8%)	Frequency of motions (61.8%) Vague pain all over the abdomen and epigastrium Flatulence and anorexia Fever
Signs	1 Involvement of caecum (31.4%) 2 Involvement of liver (8.0%) Common surgical conditions diagnosed	Only tender Chronic peptic ulcer (3.4%) Chronic appendicitis (2.5%)
<i>E. histolytica</i> and <i>G. lamblia</i> together	1 Bowel disorder (80.8%) 2 Pain (72.7%) 3 Dyspepsia (63.7%) 4 Constitutional (28.3%)	Constipation with alternate diarrhoea Iliac fossa and epigastrium Flatulence and nausea and vomits Fever and loss of weight
Signs	1 Involvement of caecum (51.5%) 2 Involvement of liver (17.2%) Common surgical conditions misdiagnosed	Palpable and tender Chronic appendicitis (5.0%) Peptic ulcer (3.0%)

Fraser and Taylor (19) and Monat and McInney (20) found frequent occurrence of diarrhoea in *G. lamblia* infection, but did not find vague pain all over the abdomen or in epigastrium as a predominant symptom. The latter

The relative incidence of amoebic and bacillary dysentery has always been a

observations have been contrary to this belief, and he found amoebic dysentery to be one and half times as common as the bacillary form. Our figures lean towards Payne's observations and it appears that the pendulum has now started to swing in the direction of amoebic dysentery again (Table VII).

TABLE VII

Investigator (Ref.)	Locality	Percentage of amoebic	Percentage of bacillary	Percentage of of a mixed infection
1 Cunningham (27)	East Bengal	11.0	85.0	
2 Vaidya (28)	Bombay	35.0	65.0	
3 Payne (21)	East India	50.7	31.6	6.0
4 Shrivastav (12)	Bombay	44.2	37.5	4.8

When comparing the relative incidence of amoebic and bacillary dysentery an important point, which may be mentioned here, is often omitted. When dysentery assumes epidemic proportions, a comparison between the two is not justifiable, because depending on the nature of the epidemic one or the other type

will naturally predominate. What is more important is to find-out the endemic prevalence of the two dysenteries in a static population, and that has been the attempt in this survey. During the period that this survey was carried out there was no recognizable epidemic or either dysentery and, therefore, the figures give their endemic incidence in the population.

The cases included under the bacillary type in the survey need a word of explanation. The criteria for the diagnosis of bacillary dysentery had been much wider and less strict than for the diagnosis of amoebic type. For amoebic dysentery the demonstration of actively motile trophozoite stage of *E. histolytica* was an essential feature, while for the diagnosis of bacillary dysentery cultural findings were not the only criterion, the cellular picture of the faecal exudate,

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Veghelyi quoted by Strong (36) found that out of 144 children infected with this parasite one fourth showed blood and mucus in stools. Brumpt, quoted by Strong (36) reported a dysenteric syndrome resembling amoebic dysentery but not ameliorated by emetine. Our findings are in agreement with the later group of workers.

Among helminths causing acute dysentery, Fleming (37) and Roger and Dammun (38) found only intractable diarrhoea, while Sangster (39) found 25 cases with blood and mucus in the stool, associated with hookworm infection. *S. stercoralis* infection causes not only chronic diarrhoea, but also acute dysentery. Levin quoted by Strong (36) in a report of 92 cases, noted mucus and blood in the stools of 5 cases, while Himman (40) found 14 cases with acute dysentery in a study of 85 cases. Role of *A. lumbricoides* in causation of acute dysentery is interesting. The two leaders among others are reported, though Faust quoted off small masses of intestinal such cases one responded while the third could not be

treated

We did not come across any *Balantidium coli* or *Isospora hominis* infection in this investigation.

The association of chronic alcoholism and presence of hepatic signs and symptoms in cases of acute amoebic dysentery is another interesting feature of this group. Da Silva (41) in 200 cases of amoebic hepatitis in Ceylon, found such

an association in 176 cases, while in our series, out of 46 cases, 17 had hepatic symptoms, in which 10 were alcoholics. This association raises three possibilities.

- (1) It may be a mere coincidental finding
- (2) Chronic alcoholism may be responsible for early cirrhotic change in the liver, but in our series, there were no other signs of cirrhosis, in form of ascites or oedema of scrotum or limbs.
- (3) Chronic alcoholism lowers the resistance of the body as a whole and of liver in particular and amoebic involvement of liver occurs with ease

This question, however, needs further investigation.

What diagnostic significance can be attached to the reaction of the stool in amoebic dysentery is difficult to say, because out of 47 samples only 6 were acidic, while 41 were alkaline to the ordinary litmus paper. To our surprise, two bacillary stools also gave an acidic reaction. Acton and Knowles (26) described clumping of red blood cells and amoeboid movement of the isolated red cells (due to the movement of adherent spirochaetes to it) in acute amoebic stool. In 46 cases of acute amoebic dysentery, clumping of red cells in nine cases and slight amoeboid movement of the outline of the red cells in three cases was found.

SUMMARY

20.2%

in this survey.

REFERENCES

- 1 Watson, J. M. (1945), *Ann. Trop. Med. Parasit.* 39: 101
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, London
p 2 Thacker

d Assoc, 13:317.
Diseases, 7th ed,

J. Neu Sci, 11: 11. 11
24
Assoc, 41: 21

A STUDY OF TRYPTIC ACTIVITY AND CHANGES IN BLOOD CONSTITUENTS IN CASES OF INTESTINAL INFESTATION

B. K. MALAVIYA AND P. R. SINDHE

From the King George's Medical College, Lucknow

It is now well known that malnutrition is a predisposing factor in chronic intestinal infestation. Chandler (1) observed that malnutrition, pregnancy, old age etc. accentuate the disease. Faust (2) explained the anaemia observed in hook worm infestation as due to deficiency in protein intake (95% of the haemoglobin molecule is derived from dietary proteins). There are two other theories for the manifestation of anaemia in hook worm disease. One takes into account the blood sucking habit of the worm while the other postulates the existence of toxic factors in the worm which depress the bone marrow. However, no correlation has been observed between the number of worms present in an individual and the drop in haemoglobin percentage nor has any toxic factor been isolated from the worm so far. In this investigation 40 cases of ankylostomiasis have been studied. Ten were severe cases in whom haemoglobin was less than 5 g %. Twenty-five cases were of moderate severity while the rest were mild in nature. In all cases routine blood examinations and serum protein estimations were carried out and the tryptic activity of the duodenal juice was determined.

RESULTS

This study showed that as the disease due to hook worm progressed serum proteins continued to decrease. Anaemia was a constant feature of severe cases which corresponded to the fall in serum proteins. Chandler (1) and Faust (2)

TABLE I

	Protein	Fat	Carbo- hydrate	Iron	Vit. A	Calories
Mild cases	85.7	8.2	472	36.4	419	2275
Moderate cases	72.8	6.2	370	31.5	370	1810
Severe cases	56.6	4.9	293	27.7	304	1379

protein. This easy explanation becomes available if one compares the tryptic activity of duodenal juice in the three groups (Table II).

TABLE II

	Haemoglobin (g)	Serum protein (g %)	Tryptic activity (units)
Mild	12.0	7.1	14
Moderate	8.8	6.5	9.3
Severe	3.0	6.0	6.5

It will be seen that the fall in tryptic activity corresponds to the fall in haemoglobin and serum protein levels. One can, therefore, surmise from the evidence given above that there is some factor in hook worm which depresses tryptic activity and thus cuts off protein intake of the body internally. Symptoms of vitamin deficiency seen in these cases are also attributable to the same cause.

REFERENCES

1. Chandler, A. C. (1929) *Hookworm disease* p. 15. MacMillan & Co. London.
2. Faust, E. C. (1949) *Human helminthology* p. 434. Lea and Febiger. Philadelphia.

THE ACTION OF DRUGS *IN VITRO* ON TAPEWORM (*HYMENOLEPIS NANA*)

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This paper describes an investigation of the action of well known chemotherapeutic compounds upon the tapeworm *Hymenolepis nana in vitro*. It was hoped that the results obtained would indicate the type of compound to which tapeworms would be susceptible.

MATERIALS AND METHODS

The worm used was *Hymenolepis nana* a common tapeworm of mice, which can be transmitted directly by ova from mouse to mouse since both stages of the worm (cysticercoid and tapeworm) take place in the same animal. The strain employed was obtained from the National Institute for Medical Research, U.K. The technique for investigating this worm in the laboratory consists of two parts.

(i) Maintenance of the infection in mice

This technique was based on that of Steward (1). Ova were obtained from mature segments of worms collected from infected mice. They are allowed to stand in water for 20 (or 44) hr. at room temperature (about 30°C). An appropriate number (500—2000) is given to mice by syringe and stomach tube. Under good conditions the percentage of mice which later contained worms was about 80 and the number of worms was 1 to 56. If only one worm was present, 21 days after infection, it was usually a large one about 5 cm. long. If many worms were present they were small 0.5–1.0 cm.

by inserting the needle of a syringe into the upper end and perfusing it with saline. If an isotonic solution buffered to be slightly acid (pH 6.8) was employed the worms seemed to come out more readily.

(ii) Exposure of worms to compounds *in vitro*

The worms recovered from the intestine were picked up free from mucus. They were then placed in 10 cc. of the compound in 10 cc. of saline per cc. and streptomycin (30–34°C). The same amount of anti-cystidase was added to each flask of 10 cc. volume were used because they happened to be available and they proved convenient. Solutions of penicillin as high as 2000 units per cc. and of streptomycin as high as 1.0 mg./cc. were harmless to the worms.

One or two worms were placed in each flask which was then bunged. Suitable concentrations of the various compounds had been previously added to

nutrient broth in each flask. Since it was desired to make a broad survey of a large number of compounds, concentrations were chosen in multiples of 10. The flasks were incubated at 37°C. For examination they were placed in a horizontal position under a binocular dissecting microscope with low magnifications where the movements of the worms could easily be observed. The worms of the control flasks with drug free medium remained actively motile for 5 days.

The action of the drug, however, was read after 24 hr. because it is unlikely that a drug given by mouth would stay in the intestine longer than this. Bacterial growth in the flask did not occur to any appreciable extent.

RESULTS

may be questioned whether they might not prove as active as mepacrine in clinical practice.

TABLE I
Effect of drugs on *H. nana* *in vitro*

No.	Compounds	1:1000	1:10000	1:100000	1:1 million	1:10 million
1	Ext. fil. cis	A	A	A	NA	
2	Stilbamidine	A	NA	NA	NA	
3	Arcoline acetarsol	A	A	A	NA	
4	Pentaquine	A	A	A	NA	
5	Pamaquin	A	A	A	NA	
6	Mel B	A	NA	NA	NA	
7	Dicestol (2,2-Dihydroxy-5,5-dichloro-diphenylmethane)	A	A	A	A	NA
8	Copper sulphate	A	NA	NA	NA	
9	Tetrachlorethylene	A	A	NA	NA	
10	Phenothiazine	A	NA	NA	NA	
11	Mepacrine	A	A	A	NA	
12	Chloroquine	A	A	A	NA	
13	Arsenamide	A	A	NA	NA	
14	Santonin	A	NA	NA		
15	Sulphadiazine	NA	NA			
16	Chloramphenicol	NA	NA			
17	Tetracycline	NA	NA			
18	Vioform	A	A	A	NA	
19	Carbon tetrachloride	A	A	NA	NA	
20	Camoforn	A	NA	NA		
21	Hetrazan	NA	NA			
22	Tartar Emetic	A	A	A	A	NA
23	Miracid	A	A	A	NA	
24	Hexylresorcinol	A	A	A	NA	
25	Piperazine	A	NA	NA		

A—Active

NA—No Action

Confirmation by *in vivo* tests

To confirm whether this *in vitro* method of testing compounds is dependable or not we carried out some *in vivo* tests with some of the more well known compounds. Large number of compounds could not be tested because of non-

uniformity of the percentage of "take" in the experimental mice. This was probably because of immunity possessed by the Institute mice as we have not as yet been able to establish a Helminth free colony of mice.

The general principles followed in these tests are those of Steward (1). A number of just weaned mice were taken and fed with 500 mature ova (kept in boiled and cooled tapwater for 20 hr at 29°C) of *Hymenolepis nana* with morphine hydrochloride, 0.05 mg mouse by stomach tube, started earlier. These animals were put in groups of five. On the 19th day random sample of faeces were taken from different groups and examined for presence of ova. Only positive groups were taken for experimental purpose. A group of five were left untreated as control. The rest were fasted for 24 hr on the 20th day and treated the next day. All including the controls were fasted for 24 hr before they were sacrificed on the 3rd day after treatment. Small intestine from each

TABLE II

No	Drug	Dose mg 20 h	No. negat	No. score	Remarks
No. treated					
1	Mepacrine	6	43	04	Very active
2	Fur sibilis	15	12	11	Active
3	D-cetol	20	25	0	Very active
4	Acetoline acetarsol	0.4	35	32	Active
5	Tetrachlorethylene	0.03 cc	31	42	Active
6	Hexylresorcinol	10	42	17	Slightly active
7	Control	—	03	322	Active

Results shown above agree reasonably well with those which we got in *in vivo* tests

numbered mouse was removed in a straight piece and washed with the help of a 20 cc syringe and blunt needle. The washing fluid used was an isotonic solution buffered to pH 6.8. The number of worms were counted and placed in 5 different groups for scoring and the score entered in book. The intestine was then placed in between two glass plates and viewed under a dissecting microscope to find out any worm left over. Scores allocated were as follows:

Very large 20 actual size $1\frac{1}{4}$ "
 Large 10 actual size about $1\frac{1}{2}$ "
 Medium 5 actual size about $\frac{1}{2}$ "
 Small $\frac{1}{2}$ actual size about $\frac{1}{4}$ "
 Very small $1/10$ actual size $1/8$ "

Discussion

In searching for new chemotherapeutic agents there is always the question whether to use *in vivo* or *in vitro* tests. Generally *in vitro* tests are much simpler but *in vivo* tests are more reliable. When the parasites are hidden away in tissues, e.g., trypinosomes or filarial worms *in vitro* tests are so unreliable that they hardly are worth doing. On the other hand when the parasites are worms lying in the lumen of the intestine *in vitro* tests seem quite justified since the conditions in the intestine are fairly similar to those in the test tube. Drugs given by mouth reach the intestine with little or no possibility of chemical change—they act upon the worm for a short time—and then they are removed by passage down the intestine for absorption. The technique which has been described for testing drugs on mouse tapeworm has the advantages of simplicity and speed. Many different drugs and concentrations can be tested in a single experiment on the

worms taken from 5-6 mice. The results are obtained in 24 hr. Only minute quantities of the compound are required for the test. And there is no need to carry out preliminary determinations of the toxicity for mice before the tests are made on the worms. Accordingly, the technique is well suited for a screening test in which large numbers of unknown chemical compounds are examined to pick out the few individuals which deserve more detailed study. We plan to apply the same technique to other intestinal worms such as *Nippostrongylus* and *Ancylostoma caninum*. It could also be easily applied to any other intestinal worms for which a source of supply can be arranged.

When a large number of compounds have been examined in this way the next step will be to make a detailed study of the selected few compounds which show a favourable ratio between their activity on the one hand and their toxicity on the other. The first step will be to test their activity *in vivo*, *i.e.*, their power to kill the worms in mice. These tests are laborious to carry out and they require

And then we should hope to arrange clinical trials which are the final criterion whether or not a new compound will be of real practical importance.

ACKNOWLEDGMENT

We wish to express our thanks to Dr B. Mukerji, Director, Central Drug Research Institute, Lucknow for the facilities provided, and for his helpful criticism and continued interest in the work.

REFERENCE

- 1 Steward, J. S. (1955), *Parasit* 45: 255.

worms taken from 56 mice. The results are obtained in 24 hr minute quantities of the compound are required for the test. And there need to carry out preliminary determinations of the toxicity for mice before tests are made on the worms. Accordingly, the technique is well suited as a screening test in which large numbers of unknown chemical compounds are examined to pick out the few individuals which deserve more detailed study. We plan to apply the same technique to other intestinal worms such as *Strongylus* and *Angiostoma caninum*. It could also be easily applied to any intestinal worms for which a source of supply can be arranged.

When a large number of compounds have been examined in this way the next step will be to make a detailed study of the selected few compounds to show a favourable ratio between their activity on the one hand and their toxicity on the other. The first step will be to test their activity *in vivo*, i.e. their ability to kill the worms in mice. These tests are laborious to carry out and they require large groups of mice since the degree of infection in individual mice is very irregular. Compounds which prove promising during *in vivo* tests will be subjected to detailed toxicity trials to make sure that they are safe to administer to mice. And then we should hope to arrange clinical trials, which are the final criterion, whether or not a new compound will be of real practical importance.

ACKNOWLEDGMENT

We wish to express our thanks to Dr B Mukerji, Director, Central Research Institute, Lucknow for the facilities provided and for his helpful criticism and continued interest in the work.

REFERENCE

- 1 Steward J S (1955) *Parasit* 45 255

STUDIES ON THE ANTHELMINTHIC ACTIVITIES OF
SEEDS OF *CARICA PAPAYA* LINN

M K KRISHNAKUMARI AND S K MUMDER

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Papaya seeds by Robinson (2) and Remando (3) have given the following results. Chemical analysis of carpine ($C_{14}H_{23}N$) alkaloid has also shown that carpine is a

also been isolated from papaya seed (7)

Papaya seed although being widely used as a herbal drug for its ascarifuge action no experimental evidence, is however available on its *in vitro* activities on helminths

There have been many attempts to maintain the nematodes outside the host in artificial cultural medium. Gavier and Savel (8) have studied the survival time of adult ascaris in a mixture of sodium chloride and sodium carbonate and also in various complex media like Ringer's and Tyrode's solutions. Robinson and Gade (9) have suggested the use of Baldwin's solution for worms for *in vitro* cultures

In the present communication the results of *in vitro* studies on the ascaricidal activities of *C. papaya* seeds on *Toxascaris transfusa*, *Ascaris* sp. and *Ascaris lumbricoides*

var *sus* are reported. Ghopra *et al* (10), Sollman (11) and others (12) have tested the action of thymol and other anthelmintics on earthworms. Due to the non availability of adequate numbers of parasitic test worms for trials on the solubility of the vermicidal agent of the seed in some solvents and studies on the stability of the drug-action on storage, earthworm (*Pheretima* sp.) was used as test organism in these experiments.

EXPERIMENTAL

Collection. Worms were obtained from the faeces of the animals in the local zoo garden which were expelled without the administration of any ascarifuge. Collection of worms was also carried out at the slaughter house. The live ones were immediately transferred to the cultural medium and was maintained at 35°C to 40°C in a vacuum flask for avoiding death due to sudden change in environmental temperature after expulsion and during transport to the laboratory. Earthworms were maintained in the laboratory on leaf mould and soil mixture.

Selection of medium. For the selection of a suitable medium for maintaining worms, preliminary studies were carried out with the following culture media:

- 1) Tyrode's solution
- 2) Ringer's Solution
- 3) Baldwin's solution
- 4) Ringer-Locke solution

On the basis of the survival time and the activity of the worms in the above mentioned media, Ringer-Locke solution was found to be the best. This was selected for the experiments. The composition was as follows:

Modified Ringer Locke solution

Sodium chloride	9.00 g
Potassium chloride	0.42 g
Calcium chloride	0.24 g
Sodium bicarbonate	0.20 g
Glucose	1.00 g
Distilled water	1000 cc

Seeds. Dried papaya (honeydew variety) seeds were obtained from a local farm and stored in sealed bottles in a refrigerator. These were powdered in a grinder and sieved through 100 mesh whenever required for the trials. Water

Toxascaris transfuga, from
Ascaris sp., from polar
 of the solution and an

incubation temperature of 34°C. *Ascaris lumbricoides* var *sus* from pigs, lived for 11 days at 34°C while *Pheretima* sp. could be maintained for 11 days at 28-29°C in the cultural medium.

results of the experiment are presented in Table 1.

TABLE I

Comparative in vitro response of test worms to papaya seed powder and piperazine citrate

Test worm	Drug	Dosage		Death time (hr)	Remarks
		Drug (g) Ringer Locke (cc)			
<i>Toxascaris transfuga</i>	Papaya seed suspension in Ringer Locke	1	50	72	Exposure to drug 18 hr Post exposure death time at 34°C in Ringer-Locke
	"	1	100	98	"
	"	1	200	122	"
	Piperazine citrate	1	50	8	"
	"	1	100	24	"
	"	1	200	60	"
	Control	0	100	236	"
<i>Ascaris lumbricoides</i> var <i>suus</i>	Papaya seed powder suspension	1	50	61	"
	"	1	100	78	"
	"	1	200	114	"
	Piperazine citrate	1	50	5	"
	"	1	100	22	"
	"	1	200	54	"
	Control	0	100	98	"
<i>Ascaris</i> sp	Papaya seed suspension	1	50	97	"
	"	1	100	131	"
	"	1	200	192	"
	Piperazine citrate	1	50	27	"
	"	1	100	103	"
	"	1	200	124	"
	Control	0	100	384	"
<i>Pheretima</i> sp	Papaya seed suspension	1	50	6	Death time in hr during exposure at 29°C in Ringer Locke medium
	"	1	100	8	"
	"	1	200	12	"
	"	1	400	28	"
	"	1	800	42	"
	Piperazine citrate	1	50	2	"
	"	1	100	3.5	"
	"	1	200	7.5	"
	Control	0	100	170	"

Further experiments were carried out using earthworms as test organisms for examining the extractibility of the active principle by some solvents and also

Results of the experiments on the solvent actions of distilled water, ammonium hydroxide, sodium bicarbonate, sodium hydroxide, hydrochloric acid solutions, ethyl alcohol, benzene, ether, carbon tetrachloride and Ringer-Locke

TABLE II

Extractibility of the vermucidal principle in papaya seed by various solvents

Test worm	Solvent	Vermucidal activity	
		Residue	Extract
<i>Toxascaris transfuga</i>	Distilled water	—	++++
<i>Pheretima</i> sp	"	—	++++
"	Dist. water after 10 mins. boiling	—	—
"	Normal saline	++	++
"	Ringer-Locke	++	++
"	Sodium bicarbonate (0.5%)	+++	+
"	(1.0%)	++	++
"	Sodium hydroxide (0.05%)	+++	++
"	Ammonium hydroxide (5%)	+	+++
"	Hydrochloric acid (1%)	—	—
"	Ethyl alcohol	++++	—
"	Acetone	++++	—
"	Ether	++++	—
"	Benzene	++++	—
"	Chloroform	++++	—
"	Carbon tetrachloride	++++	—

TABLE III

Stability of vermucidal principle in papaya seed on storage

	Storage condition Temp	RH (%)	Period (days)	Percent mortality on 5 hr exposure in papaya seed 5 mg/cc of Ringer- Locke
Seed powder	24°-29°C	68-75	0	100
"	24°-29°C	68-75	15	0
"	60°C		2	30
"	26° Vacuum		15	100
"	24°-29°C		30	70
"	Air filled at atmospheric pressure		15	70
"	24°-29°C		30	50
"	Under CO ₂		15	80
"	24°-29°C		30	80
"	Under Nitrogen		15	100
"	24°-29°C		30	70
"	Water extract freeze dried loosely stoppered		15	60
"	24°-29°C	68-75	30	10
"	Freeze dried		15	90
"	water extract and stored under 26° vacuum		30	80
"			180	70
Stored as seed	24°-29°C	68-75	60	100
"	29°C	90	180	100
"			60	80
"			180	50
Stored as powder	29°C	30	15	60
"	29°C	90	15	0

RH = Relative Humidity.

medium on the papaya seed vermucidal principle are presented in Table II. Extracts of papaya seed were prepared by shaking the powdered seed with solvents on a reciprocating mechanical shaker for 4 hr and the organic solvents were vaporated at room temperature under vacuum. Alkaline and acid extracts were neutralized before use. The extracts were tested for their vermucidal activities on the *Pheretima* sp. The residues left after extraction of the powdered seeds with various solvents were also tested for their vermucidal potencies on their water extracts.

Freeze-dried water extract of papaya seed powder was prepared and used in the experiment for its stability of the vermucidal activity on storage. Results of the experiment on the stability of the active principle of the seed on storage under nitrogen, carbon dioxide, vacuum and air are given in Table III.

DISCUSSION

Results of the *in vitro* experiments revealed that papaya seeds contained a principle cidal to *Toxascaris transfiga*, *Iscares lumbricoides* var *mus* and *Pheretima* sp. *Ascares* sp. from the polar bear was found to be relatively resistant to the action

the yield of extractive from papaya seed powder with normal saline solution, water and Ringer's solution. It was observed that higher yield was obtained with distilled water alone in comparison with water containing the solutes such as sodium chloride, calcium chloride and potassium chloride. This factor was probably responsible for the higher activity of the papaya seed in the test where water extract of the seed was used (vide Table II). However, this requires further verification.

Experiments on the solubility of the vermucidal principle of Papaya seed in

before extraction with the organic solvents. Distilled water and dilute alkalis extracted the active principle from the papaya seed. The residues left after extraction with distilled water and alkalis showed no or very little vermucidal activities (Table II). The extracts obtained from the water and alkalis after drying showed very high order of vermucidal actions which were indicated by the response of *Pheretima* sp. in the test medium.

Thermolability of the active principle was demonstrated in the experiment (Tables II and III). Air and moisture reduced the vermucidal activity of papaya seed powder on storage. The whole seed maintained its vermucidal activity for even six months without detectable loss at room temperature (24°C to 29°C). Papaya seed powder stored well under vacuum, nitrogen and CO₂.

ACKNOWLEDGMENT

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REFERENCES

- 1 Kirtane V D and B D Chatterjee *Indian J. Chem.* **1966** *4* 157
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12

STUDIES ON SOME OF THE EFFECTS OF *ASCARIS LUMBRICOIDES* IN VITRO AND IN VIVO

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Besides the mechanical obstructions brought about by *Ascaris lumbricoides* (round worm) in the various parts of the body which may bring about a mild derangement of the function of the organ wherein they may lie, or may bring about an acute surgical emergency these worms have been known to adversely affect the system of the host in many ways. Their mere presence inside the body may make the host manifest a vast variety of allergic phenomena or may bring about disease in diverse ways.

They are also known to elaborate antitryptic substance for their survival in the host's intestines (1). This substance may interfere with the protein uptake

getting ascaris did not grow as fast as the controls even though the diet was almost the same and contained all the vitamins, salts and proximate constituents in balanced proportions. Further feeding experiments were undertaken. In these later experiments higher amounts of ascaris were fed to the rats and in one group milk proteins were omitted.

Rats were killed after sometime to see whether there was any macroscopical or microscopical changes in their viscera due to eating ascaris meat.

Experiments were performed on the dogs to see if there was any general effect of the fresh human ascaris on blood pressure and respiration. These effects were studied with total extracts, filtered extracts and deproteinized extracts. Attempt was specially made to compute the quantity of worms which would cause deleterious effects.

Ascaris were also tested for anti-enzyme activity *in vitro* and finally, the rats were tested for allergy to see whether or not the adult ascarids whose normal

For testing the anti enzyme activity individual washed worms were ground in a porcelain pestle and mortar with cleaned and sterilized sand at 0°C and extracted in normal saline to give a dilution of 1:10. Anti trypsin, anti amylase and anti lipase activities were measured according to standard methods.

Standard enzyme solution was taken to be the juice obtained from pancreatic fistulae made in dogs. Enzymic activity of this juice was studied within 15 min. of its collection and was accepted as standard. Any depressing effect on this activity *in vitro* at 37°C in one hour due to the ascaris extract, was studied and quantity inactivation of the pancreatic juice by one worm computed.

It was expected that if the ascaris does bring about allergic reactions, feeding

the worm tissues
kept for about
d by grinding a
(fresh weight)
red by grinding
was either cloth
ated by using
made up to the

required dilution just before the experiment. In all the cases, the extract was injected in the femoral vein of a dog and the dose in cc. was the same as the weight of the dog in kg.

OBSERVATION AND RESULTS

It was found that the weights increased equally in both the groups, *i.e.*, approximately 20 g. per rat for the first 11 days when they were both fed with the control diet. They increased equally for another 4 days after the introduction of the experimental diet and subsequently the increase was slower in the ascaris-fed group. Thus on the 72nd day, the ascaris fed rats had gained 79 g. on an average, whereas the controls had gained 97 g. per rat.

Ascaris meat was included in the diet of the experimental animals of a second group and the weight increase pattern was the same as in the earlier animals. The ascaris fed rats gained on an average 23 g. in 14 days, and 77 g. in 64 days (53 days after commencement) whereas the controls gained 23 g. and 96 g. respectively.

When 20% ascaris meat was included in the diet of the experimental animals the weight increase pattern was the same again as in the animals of group 1 and 4. The ascaris fed rats gained, on an average 23 g. in 14 days, and 77 g. in 64 days (53 days after commencement) whereas the controls gained 23 g. and 96 g. respectively.

In a next batch of rats 20% of ascaris meat was fed but the proteins of the skimmed milk were replaced by wheat proteins.

Thus, in a next batch of rats 20% of ascaris meat was fed but the proteins of the skimmed milk were replaced by wheat proteins. The ascaris fed rats gained, on an average 23 g. in 14 days, and 77 g. in 64 days (53 days after commencement) whereas the controls gained 23 g. and 96 g. respectively.

As mentioned earlier the effects of extracts of ascaris were studied systematically. The following Figs 1, 2 and 3 record the effects of total ascaris extract, filtered extract and deproteinized extracts.

Those tracings show that there is a definite fall in blood pressure on injection of ascaris extract into the dogs, the pressure, however, returns to normal after a variable period if the dose is not too large. The effective dose goes on increasing as the protein content of the extract decreases. Thus 1/40 of the total extract caused a recoverable fall and 1/20 caused a fatal fall in blood pressure.

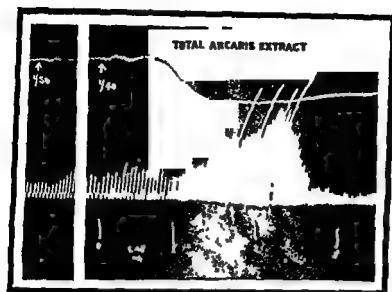


Fig. 1

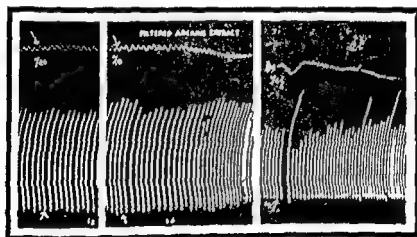


Fig. 2

With a filtered extract the fall in weight of the rat with the deproteinated extract only was effective (by 100 mg per day) and then also the effect was reversible.

There was almost no effect in any rat.

Macroscopical and microscopical changes

There were no changes at all in the weight of the rat in the liver

TABLE I
Anti tryptic activity

Worm no	Weight (g.)	Pancreatic juice inactivated (%)	Pancreatic juice inactivated per g. (cc)	Pancreatic juice inactivated per worm (cc)
1	0.9	37.5	3.75	3.4
2	1.3	100.0	10.00	13.0
3	1.25	33.7	3.37	4.2
4	2.3	81.6	8.16	18.8
5	1.65	65.9	6.59	10.9
6	3.5	92.1	9.21	32.2
7	2.2	40.9	4.99	10.9
8	1.3	6.6	0.66	0.9
9	2.4	57.7	5.77	13.8
10	1.5	87.5	8.75	8.75
11	3.4	78.6	7.86	26.7
12	2.55	75.0	7.5	19.2
13	3.0	27.7	2.77	8.1
14	1.3	36.9	3.69	4.8
15	2.4	23.1	2.31	5.5
16	7.0	36.9	3.69	25.8
17	3.7	21.5	2.15	7.9
18	2.5	15.4	1.54	3.8
19	1.7	24.3	2.42	3.7
20	2.1	43.4	4.24	8.9
21	0.9	15.1	1.51	1.4

Average weight of the worm 2.33 g. average percentage of inactivation 48.1, average volume of pancreatic juice inactivated by 1 g. of ascars 4.81 cc. average volume of pancreatic juice inactivated by 1 worm 11.3 cc.

1930, the appearance of parenchymal degeneration of the liver varying from mild to severe and the interlobular veins in dogs, guinea pigs, and rats. He ascribed these changes to the presence of such toxins in the adult worms.

used in our experiments. It is possible that Blackie's results were due to allergens and not due to toxins. It may be specially noted that the intestinal mucosa of the ascaris-fed animals was also normal.

There is some substance present in the ascaris which lowers the blood pressure. The effects are qualitatively same in all the 3 groups though, quantitatively, deproteinized extracts have only 1/20th effect of the total extracts. These effects are qualitatively and quantitatively similar to those described by Emery and Herrick (6) and others. They ascribed these changes to the presence of an albumose-peptone, named 'askaron' by Shimamura and Fujii (7). Similarly, Iino (8), reported the toxic effects of the body cavity fluid of the ascaris.

The retardation in the growth has been extremely pronounced in the rats fed on milk free diet where it amounts to 72% of the normal growth even though substitution of skimmed milk powder by wheat flour reduced the protein content by a bare 2%. It is possible that protein content of the standard diet was marginal and just sufficient to keep the growth rate at a tolerable level. In the presence of anti tryptic activity of the ascaris and a slight reduction in the protein intake has tipped the scales against the animals and the growth has come to a very low level.

More probably, it is the quality of the protein which has produced these effects. Shorb and Spindler (15) have reported that skimmed milk diet fed to young pigs infested with ascaris resulted in good weight gains since the host eliminated the parasites automatically. Kozami (16) also reported that pigs which were fed on milk and plenty of cod liver oil could not be experimentally infected with ascaris larvae. It has been reported that malnutrition *per se* (even without intestinal parasites) reduces the tryptic properties of pancreatic juice presumably these properties would return to normal if malnutrition is corrected. It would appear that milk proteins have some unexplained effects on the anti tryptic properties of ascaris which not only protect the host from the injurious effects of ascaris already present but actually help him to eliminate the parasites and regain proper nutriture at a still faster rate or even to resist the infection gaining a foothold.

SUMMARY

An attempt has been made to spot out anti nutritive or toxic factors in the body of adult *Ascaris lumbricoide*. Protein free extracts of the worms had in high concentrations a mild reversible depressant effect on the dog's blood pressure.

The growth of rats was retarded by including fresh ascaris meat in their normal diet. Exclusion of milk from the normal diet of these rats retarded weight gain further.

Ascaris feeding for long periods did not have any sensitizing effect on the rats.

Ascaris extracts were found to suppress tryptic activity of the pancreatic juice *in vitro*.

Except for a slight liver enlargement no significant changes were noted macroscopically or microscopically in the viscera of rats kept on high ascaris diet.

REFERENCES

1. Wenland E. (1903) *Ztschr F Biol* 44: 1 quoted by Baeding H. (1949) *Physiol Rev* 29: 211.
2. Farbar D. (1957) *Exptl Parasit* 6: 494.
3. Ikuji K. (1926) quoted by Venkatachalam H. S. and Patvardhan R. (1953) *Trans Roy Soc Trop Med Hyg* 47: 169.
4. ...
5. ...
6. ...
7. ...
8. ...
9. ...
10. ...
11. ...

- 12 Morisita, T and Babuchi, M (1957), *Japanese J Exptl Med*, 27: 1, quoted in *Trop Dis Bull*, (1951), 48: 383
- 13 Patwardhan & Venkatchalam, I S M R 1953
- 14 Sindhe, P R, personal communication
- 15 Shorb, D A and Spindler, I A (1947), *Proc Helm Soc Wash*, 14 30, quoted by Venkatchalam *et al*, *loc cit*
- 16 Kiodzami, M (1927), *Japan Med World*, 7 12, quoted in *Trop Dis Bull* (1927), 24: 997.
- 17 Malaviya, B K and Sindhe, P R, this Symposium

CONCLUDING REMARKS

DR H B BHATIA

At the outset, I would like to congratulate Dr Mukerji for having selected the topic of amoebiasis for this year's symposium which is of national importance to our country, and on his having collected workers of international fame in this field for discussion on its chemotherapy.

That this disease has high incidence and is difficult to treat in the chronic form is evident from the fact that 50% of the patients who came to my consulting room complain of abdominal symptoms and 75% of them turn out to be suffering from chronic amoebiasis. During the last two years 396 stool specimens were

Dr Srivastava in the city of Bombay

Dr Srivastava has reported incidence of 20% infection in healthy individuals. From another paper, which you have just heard, varied clinical forms of this disease have been discussed. It is thus obvious that thorough research is necessary to determine the pathogenesis of the various clinical syndromes. To what

weight, low blood pressure, some in which anxiety neurosis has developed during the course of this illness. As regards the extra intestinal forms I have seen two cases of primary lung abscess, both of which were cured after emetine injections. I have also seen three cases of serious type of pleural effusion secondary to an hepatitis, in which both the hepatitis and effusion were cured with emetine.

As regards therapeutics I still consider emetine by far the most useful drug in the treatment of acute and extra intestinal form. For the treatment of chronic amoebiasis, numerous drugs have flooded the market in recent years and an to choose. The superiority of each onally I like to stick to old remedies. In giving relief to my patients drug, my patients have come back

to me saying that my first prescription was better.

I, therefore, feel that no outstanding advance has been made for the treatment of chronic stages. In order to judge the clinical adequacy of new compounds, it would be most important to distinguish between relapses and reinfections, as long as patients are living in the same unhygienic environments chances of reinfections are there. It would thus again be necessary to carry out therapeutic trials for long periods in special wards where chances of reinfections are obviated to see the effect of drug in producing permanent cures. At present, my treatment of chronic cases consists of giving 5 days' course of camoform tablets (1 tablet four times a day) followed by one month's treatment with enterovioform (1 tablet

thrice a day) I very often give tablets of bellargol and yeast with enterovioform and with this line of treatment nearly all my cases are relieved. Some of them, who went abroad to U K. or U S A. for a couple of years after this treatment have remained well, whereas many of those living in the old un hygienic environments came back with recurrence of symptoms after lapse of six months or a year. However, we must keep our eyes wide open, and if some really superior drug is found for chronic cases it will prove a great boon to us.

You have listened to some very interesting papers this morning, and I am sure everyone of you would like me to thank the speakers on your behalf and my own for the trouble they have taken in coming over from very long distances to take part in this very important symposium. I also thank everyone of you for the co operation you have given, which has enabled me to run the papers according to the time schedule.

